EPA Registration Jacket 43813-27 Vol. 3

Material to be added to an e-Jacket/Jacke

Reg. No. 43813-27

1. ☑ Placement within the e-Jacket/jacket:
   - ☐ Default: (chronological, top/newest)
   - ☐ Description: (PDF page number, i.e., "before page 45")

2. ☐ Send to Data Extraction contractors this material:
   - ☐ Newly stamped accepted label
   - ☑ Notification
   - ☐ New CSF
   - ☐ Other: _____

3. Attach this coversheet to the top of the material or jacket, must be well organized and clipped together, NOT STAPLED Then give the material with this coversheet to staff in the Information Services Center (Room S-4900).

Reviewer's Name: M Terry

Phone: 308-6217 Division: AD

Date: 9/11/09

Created July 21/

mact

DECISION PKG. NO. 420336 SUBM. DUE DATE 9/13/09

SUBMISSION BAR CODE # 858362 REVIEWER MT

CODING FORM FOR APPLICATIONS FOR REGISTRATION/AMENDMENTS

FILE SYMBOL/REG NO. 43813-27 PM 33 ACTION CODE 332 PRIA

DESCRIPTOR NOTIFICATION FQPA NFQPA

[ ] CHILD RESISTANT PACKAGING: [ ] REQUIRED [ ] NOT REQUIRED

REGISTRATION TYPE: [ ] CONDITIONAL [ ] UNCONDITIONAL [ ] RESTRICTED USE

DATE ON APPLICATION	EPA RECEIVE DATE	PM RECEIVE DATE
8/10/09	8/13/09	8/13/09

METHOD OF SUPPORT

[ ] CITE-ALL [ ] SELECTIVE
[ ] NOT SUBMITTED [ ] N/A

FORMULATORS EXEMPTION

[ ] SUBMITTED [ ] NOT SUBMITTED
[ ] N/A

REVIEW(S) REQUESTED	DATA PACK #	DATE SENT	DUE DATE	DATE RETURNED
CHEMISTRY				
EFFICACY				
ACUTE TOX.				
RASSB TOX.				
ENVIRON. FATE				
FISH/WILDLIFE				
OTHER:				

STATUS

RESPONSE CODE 1153 RESPONSE DATE 9/11/09

SEP 11 2009

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

EPA United States Environmental Protection Agency **Office of Pesticide Programs**

Janssen PMP
a Division of Janssen Pharmaceutical NV
1125 Trenton-Harbourton Road
Titusville, N. J. 08560-0200

Attention: William R. Goodwine, Director
Regulatory Affairs and Product Development

Subject: ECONEA Technical
EPA Registration No. 43813-27
Notification Dated August 10, 2009

This will acknowledge receipt of your notification of label change per PR Notice 2007-4 and PR Notice 83-3, submitted under the provisions of FIFRA Section 3(c)(9). Based on a review of the submitted material, the following comments apply.

The Notification dated November 4, 2008 is in compliance with PR Notice 98-10 and is acceptable. This information has been added to your file.

If you have any questions concerning this letter, please contact Martha Terry at (703) 308-6217.

Sincerely,

Martha Terry /for

Marshall Swindell
Product Manager (33)
Regulatory Management Branch 1
Antimcrobials Division (7510P)

Please read instructions on reverse before completing form.

Form Approved. OMB No. 2070-0060

United States
Environmental Protection Agency
Washington, DC 20460

[ ] Registration
[ ] Amendment
[X] Other

OPP Identifier Number

Application for Pesticide - Section I

1. Company/Product Number	2. EPA Product Manager	3. Proposed Classification
43813-27	Marshall Swindell	[X] None [ ] Restricted
4. Company/Product Name	**PM#**	
ECONEA Technical	33	

5. Name and Address of Applicant (Include ZIP Code)

Janssen PMP, a Division of Janssen Pharmaceutica, N.V.
1125 Trenton-Harbourton Road
Titusville, NJ 08560-0200

[ ] Check if this is a new address

6. Expedited Review. In accordance with FIFRA Section 3(c)(3)(b)(i), my product is similar or identical in composition and labeling to:

EPA Reg. No. _____

Product Name _____

Section - II

[ ] Amendment - Explain below.
[ ] Resubmission in response to Agency letter dated _____
[X] Notification - Explain below.
[ ] Final printed labels in response to Agency letter dated _____
[ ] "Me Too" Application.
[ ] Other - Explain below.

Explanation: Use additional pages if necessary. (For section I and Section III.)

"Notification of label change per PR Notice 2007-4. This notification is consistent with the guidance in PR Notice 2007-4 and the requirements of EPA's regulations at 40 CFR 156.10, 156.140, 156.144, 156.146, and 156.156. No other changes have been made to the labeling or the Confidential Statement of Formula for this product. I understand that it is a violation of 16 U.S.C. Sec. 1001 to willfully make any false statement to EPA. I further understand that if the amended label is not consistent with the requirements of 40 CFR 156.10, 156.140, 156.144, 156.146, and 156.156, this product may be in violation of FIFRA and I may be subject to enforcement action and penalties under sections 12 and 14 of FIFRA."

Section - III

1. Material This Product Will Be Packaged In:

Child-Resistant Packaging	Unit Packaging	Water Soluble Packaging	2. Type of Container
[ ] Yes [X] No	[ ] Yes [X] No	[ ] Yes [X] No	[ ] Metal [ ] Plastic [ ] Glass [ ] Paper [XXXXX] Other (Specify) plastic lined fiber drum
* Certification must be submitted	If "Yes" Unit Packaging wgt. / No. per container	If "Yes" Package wgt / No. per container	

3. Location of Net Contents Information	4. Size(s) Retail Container	5. Location of Label Directions
[X] Label [ ] Container	110 lbs (50 kg)	On Label

6. Manner in Which Label is Affixed to Product

[XXXXX] Lithograph
[ ] Paper glued
[ ] Stenciled
[ ] Other _____

Section - IV

1. Contact Point *(Complete items directly below for identification of individual to be contacted, if necessary, to process this application.)*

Name	Title	Telephone No. (Include Area Code)
William R. Goodwine	Senior Director, Regulatory Affairs & Product Development	(609) 730-2607

Certification

I certify that the statements I have made on this form and all attachments thereto are true, accurate and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

6. Date Application Received (Stamped)

2. Signature	3. Title
William R. Goodwine	Senior Director, Regulatory Affairs & Product Development
4. Typed Name	**5. Date**
William R. Goodwine	August 10, 2009

EPA Form 8570-1 (Rev. 3-94) Previous editions are obsolete. White - EPA File Copy (original) Yellow - Applicant Copy

ECONEA® Technical

Anti-fouling Preservative

For Formulating Use Only

ACTIVE INGREDIENT:	
Tralopyril*.....	93.2%
INERT INGREDIENTS:	6.8%
TOTAL:	100.0%

* CAS# 122454-29-9

KEEP OUT OF REACH OF CHILDREN

DANGER POISON

See side panel for first aid and additional precautionary statements

EPA Reg. No.: 43813-27

EPA Est. No.: 241-MO-001

Manufactured for: **JANSSEN PMP**
a Division of Janssen Pharmaceutica NV
1125 Trenton-Harbourton Road
Titusville, NJ 08560

® Registered Trademark of Janssen Pharmaceutica

Rev. 08/09

PRECAUTIONARY STATEMENT
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

DANGER

Fatal if swallowed. Harmful if inhaled or absorbed through the skin. Causes moderate eye irritation. Avoid breathing dust. Avoid contact with skin, eyes, or clothing. Wash hands thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. Remove contaminated clothing and wash clothing before reuse.

Handler Personal Protective Equipment (PPE):

- Wear long-sleeved shirt, long pants, socks, shoes, and chemical resistant natural rubber gloves.
- Wear protective eyewear such as goggles, face shield or safety glasses.
- Wear dust filtering respirator (MSHA/NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N, R, P, or HE filter.

Pesticide User Safety Requirements:
Discard clothing and other absorbent materials that have been heavily contaminated with this product. Do not reuse them. Follow the manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

FIRST AID	
If **swallowed**	-Call a poison control center or doctor immediately for treatment advice. -Have person sip a glass of water if able to swallow. -Do not induce vomiting unless told to do so by a poison control center or doctor. -Do not give anything by mouth to an unconscious person.
If **inhaled**	-Move person to fresh air. -If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. -Call a poison control center or doctor for further treatment advice
If in **eyes**	-Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. -Call a poison control center or doctor for treatment advice.
If on **skin** or **clothing**	-Take off contaminated clothing. -Rinse skin immediately with plenty of water for 15-20 minutes. -Call a poison control center or doctor for treatment advice.

HOT LINE NUMBER:

For information on this pesticide product (including health concerns, medical emergencies, or pesticide incidents) call the National Pesticide Telecommunications Information Center at 1-800-858-7378.

For chemical emergency assistance (spill, leak, fire, or accident), call Chem Trec at 1-800-424-9300.

Have the product container with you when calling a poison control center or doctor, or going for treatment.

NOTE TO PHYSICIAN

Probable mucosal damage may contraindicate the use of gastric lavage.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. This product is intended for use only during industrial formulation processes producing antifouling products for control of hard fouling organisms. Formulators using ECONEA are responsible for providing additional data to support registration of their end-use product(s).

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage and disposal.

STORAGE: DO NOT mix or store this product or solutions of this product in a manner inconsistent with its labeling.

PESTICIDE DISPOSAL: Pesticide wastes may be acutely hazardous. Improper disposal is a violation of Federal Law. Pesticide, mixtures, or equipment rinse waters that cannot be chemically reprocessed must be disposed of according to applicable federal, state or local procedures. Contact your State Pesticide or Environmental Control Agency or the Hazardous Waste representative at the nearest EPA Regional Office for guidance.

CONTAINER DISPOSAL: Non-refillable container. Do not reuse or refill this container. Offer for recycling, if available. Completely empty liner by shaking and tapping sides and bottom to loosen clinging particles. Empty residue into formulation equipment. Then dispose of liner in a sanitary landfill or by incineration if allowed by State and local authorities. If burned, stay out of smoke. If drum is contaminated and cannot be reused, dispose of in the same manner.

ENVIRONMENTAL HAZARDS

Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA.

NOTICE OF WARRANTY

Janssen PMP, a Division of Janssen Pharmaceutica NV warrants that this product conforms to the chemical description on the label thereof and is reasonably fit for purposes stated on such label only when used in accordance with the directions under normal use conditions. It is impossible to eliminate all risks inherently associated with the use of this product. Ineffectiveness or other unintended consequences may result because of such factors as weather conditions, presence of other materials, or the manner of use or application, all of which are beyond the control of Janssen PMP. To the extent permitted by law, Janssen PMP disclaims any liability for consequential, special or indirect damages resulting from the use, handling, application, storage or disposal of this product or for damages in the nature of penalties, and the buyer and user waive any right that they may have to such damages. To the extent consistent with applicable law, Janssen PMP makes no warranties of merchantability or of fitness for a particular purpose or any other express or implied warranty except as stated above.

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** Econea - degradate **PC Code No.:** N/A (metabolite of 119093)

2. **TEST MATERIAL:** CL322,248 **Purity:** 94.5%
CAS No.: Not listed
Batch No.: 1547-24

3. **CITATION**

Author:	Lee E. Sayers
Title:	CL322,248 – Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) under Static Conditions
Study Completion Date:	January 24, 2006
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6159 Janssen Study No. AGR 1178
MRID No.:	467513-09

4. **REVIEWED BY:** Kathryn Montague, M.S., Biologist RASSB/AD/US EPA
Signature: Kathryn V. Montague **Date:** 09/19/06

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader RASSB/AD/US EPA
Signature: Siroos - Mostaghi **Date:** 9/20/06

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	Sheepshead Minnow (*Cyprinodon variegatus*)
Age of Test Organism:	Not reported
Definitive Test Duration:	96 hours
Study Method:	Static

Type of Concentrations: Mean measured

7. **CONCLUSIONS**

Verified Results Synopsis: 96-hr LC_{50}: > 89 mg a.i./L 95% C.I.: Could not be calculated.
NOEC: 89 mg a.i./L

8. **ADEQUACY OF THE STUDY**

A. Classification: Acceptable

B. Rationale: Although there are deviations from Guideline recommendations, they did not affect the results of the study

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS:**

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- The study report does not indicate if fish were used in previous tests or if there were signs of stress or injury prior to testing.
- The study report does not indicate whether disease treatments were administered within 48 hours of test initiation or during the exposure period.
- No information was provided regarding colors or light intensities during the acclimation period.
- The study report states that pesticides, PCBs and toxic metals were not detected in the dilution water at concentrations considered toxic; however, actual concentrations of these compounds are not reported.
- The guideline states that the pH of the test solutions should be > 7.5 and < 8.5 for marine testing. During the study period, the pH ranged from 7.1 to 7.9.
- The study report does not indicate if the test chambers were covered during the test. The guideline states that test concentrations should be selected to produce a NOEC and, preferably, at least 2 partial mortalities (> and < 50%) after 96 hrs. No mortality was observed during the exposure period at any of the test concentrations. An attempt to determine the LC50 by increasing dose levels was not made, since the highest treatment level approximated the water solubility limit of the test substance.
- Test substance concentrations were not measured in each individual replicate. At test initiation, samples were taken from the intermediate mixing vessel prior to division into replicate vessels. At test termination, samples for concentration analysis were removed from a composite of replicates A and B. Therefore, variations in test substance concentrations between replicates of each dose level could not be determined.

- The guideline indicates that the test substance concentration should be measured at the beginning, at 48 hours, and at the end of the test. No concentration analysis was conducted at 48 hours since preliminary testing indicated that the test substance was stable in the marine matrix for over 96 hours.
- The study report does not state whether fish were added to the test vessels within 30 minutes of addition of the test substance, as stipulated by the guideline.

10. **SUBMISSION PURPOSE:** Registration of the parent compound, "Econea" (R107894, aka CL303268) for antifouling use.

11. **MATERIALS AND METHODS**

A. Test **Organisms**

Guideline Criteria	Reported Information
Species ▪ Preferred freshwater species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*) ▪ Preferred saltwater species: Atlantic silverside (*Menidia menidia*)	• Sheepshead Minnow (*Cyprinodon variegatus*)
Weight ▪ Juvenile fish < 3.0 g	• Mean wet weight = 0.16 g (range: 0.09 to 0.24 g) (p. 8)
Length ▪ Longest not > 2x shortest	• Longest less than 2 times the shortest; total length range: 18 to 24 mm (mean total length: 21 mm) (p. 8)
Supplier	• Aquatic BioSystems, Inc., Fort Collins, Colorado. (p. 11)
All fish from same source and population?	• Yes. (p. 11)
Fish used in previous tests?	• Not discussed in the study report, but not likely due to age of fish.
If wild fish used, quarantined 7 days before acclimation?	• Not applicable
Signs of stress or injury?	• Not discussed in the study report.

B. Acclimation

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Acclimation Period • Minimum 12 days (14 days recommended) • Minimum 7 days in test dilution water	• 14 days. (p. 11)
Holding Water • Same source as test dilution water (if not, acclimation to dilution water done gradually over 48 hr period)	• Yes. (pp. 11-12)
Disease Treatment • No treatments within 48 hrs of test initiation or during test	• Not discussed in the study report.
Feeding • No feeding within 48 hrs of test initiation. Feed daily prior to this period.	• Fish fed daily except during the 48 hour prior to testing and the 96-hr definitive exposure period. (p. 11)
Pretest Mortality • < 5% during acclimation; reject entire batch if > 10%.	• No mortality was observed during the 48-hr period prior to testing. (p. 12)
Water Temperature • Temperature changes should not exceed 3°C per day • Hold fish minimum 7 days at test temperature prior to testing	• Temperature in holding tank ranged from 22 to 23 C, same as test temperature. (pp. 11 and 17) • Fish held in holding tank at test temperature for 14 days (p. 11)
Background • During final 48 hrs, colors and light intensities similar to testing area	• Not discussed in the study report.

C. Test System

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Dilution Water ▪ Reconstituted water or water from natural source preferred. If dechlorinated tap water, daily chlorine analysis performed. ▪ Chemical analysis performed and maximum concentrations not exceeded (see guideline)	• Dilution water used in the study was filtered natural seawater diluted with laboratory well water. (p. 12) • Dilution water analyzed periodically for the presence of pesticides, PCBs and toxic metals. According to the study report, none of these compounds were detected at concentrations considered toxic; actual concentrations not reported. (p. 12) • TOC < 2.0 mg/L (p. 12)
Solutions ▪ Distilled water used to make stock solutions of test substances. If stock volume > 10% of test solution volume, dilution water used.	• Each exposure solution was prepared by adding the appropriate amount of test substance and 2 mL of acetone to 1 L of dilution water. (p. 13)
Water Temperature ▪ 10 or 12 ± 2°C for cold water species (see guideline) ▪ 22 or 23 ± 2°C for warm water species (see guideline) ▪ Vary no more than 1°C in any 24-hr period ▪ Record in all replicates at beginning of test and every 24 hrs; record hourly in one replicate.	• Solution test temperature ranged from 22 to 23 °C during the exposure period. (pp. 17 and 21) • Temperature was measured once daily in all treatment and control aquaria. Test temperature was continuously monitored in replicate A of the 25 mg a.i./L solution. (p. 15)
pH ▪ > 6.0 and < 8.0 for freshwater testing ▪ > 7.5 and < 8.5 for marine testing ▪ Measured in each replicate at beginning of test and every 24 hrs	• pH ranged from 7.1 to 7.9 during the exposure period. (p. 21) • pH was measured once daily in all treatment and control aquaria. (p. 15)
Dissolved Oxygen ▪ Static: > 60% saturation at all times ▪ Flow-through: > 75% saturation at all times ▪ Measured in each replicate at beginning of test and every 24 hrs	• Dissolved oxygen concentration was above 60% saturation throughout the exposure period. (pp. 17 and 21) • Dissolved oxygen was measured once daily in all treatment and control aquaria. (p. 15)
Total Hardness ▪ 40 to 180 mg/L as $CaCO_3$ (freshwater species) ▪ Measured at beginning of each test	• Not applicable.

Guideline Criteria	Reported Information
Salinity ▪ 20 ± 5ppt (estuarine species) ▪ Measured at beginning of each test and, for flow-through tests, on day 4, and if extended days 7 and 14	• Salinity ranged from 19 to 21 ppt during the exposure period. (p. 21) • Salinity was measured once daily in all treatment and control aquaria. (p. 21)
Test Aquaria/Equipment ▪ Material: Glass, stainless steel, nylon screen or perfluorocarbon plastic (e.g., Teflon®) ▪ Test chambers loosely covered	• Glass aquaria (15 x 15 x 30 cm). (p. 13) • No cover is mentioned in the report.
Aeration ▪ Static systems only if < 60% saturation; if aeration used test concentrations measured. ▪ No aeration in flow-through tests	• Dissolved oxygen concentration dropped to 64% saturation in one replicate at 72 hours; gentle aeration was initiated to maintain dissolved oxygen concentrations above 60% for the remainder of the exposure period. (p. 17) • Exposure solutions were analyzed at test initiation and test termination; measured concentrations were consistent between sampling intervals and maintained expected concentration gradient. (pp. 15, 17, and 22)
Type of Dilution System ▪ Must provide reproducible supply of toxicant	• Not applicable.
Flow Rate ▪ Consistent flow rate of 6-10 vol/24 hours ▪ Measured at beginning and end of each test ▪ No more than a factor of 10 variation between replicates	• Not applicable.
Biomass Loading Rate ▪ Static/Static-renewal: ≤ 0.8 g FWF/L ▪ Flow-through: ≤ 0.5 g FWF/L	• 0.23 g of biomass/L. (p. 14)
Photoperiod ▪ Range from 12D/12N to 16D/8N, with 15 min transition period ▪ Intensity 30 to 100 lm at water surface	• 16 hours light/ 8 hours darkness; transition period not specified. However, study report noted that sudden transitions were avoided. (pp. 11 and 13) • Intensity at the solutions' surface ranged from 75 to 93 footcandles (810 to 1000 lux). (p. 13)

Guideline Criteria	Reported Information
Solvents ▪ Not to exceed 0.5 ml/L for static or static-renewal tests or 0.1 ml/L for flow-through tests ▪ Preferred solvents dimethyl formamide, triethylene glycol, methanol, acetone, or ethanol	• Acetone was used as a solvent at a concentration of 0.1 ml/L. (p. 13)

D. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • If LC_{50} > 100 mg/L with 30 fish, then no definitive test required	• Range-finding test conducted at concentrations of 0.010, 0.10 and 1.0 mg a.i./L (solvent control: dimethylformamide). (p. 16) • No mortality or adverse effects observed at any treatment level or the control. (p. 16)
Test Concentrations • Minimum of control and 5 concentrations in geometric series • Concentrations 50 to 120% greater than next lowest concentration • No more than 25% variation between test concentrations within same treatment • Concentrations selected to produce NOEC and, preferably, at least 2 partial mortalities (> and < 50%) after 96 hrs • Measured concentrations required if test chemical unstable or flow-through system, and must remain at least 80% of nominal concentrations	• Control and five concentrations used (6.3, 13, 25, 50 and 100 mg a.i./L). (p. 13) • Each concentration level was approximately 50% greater than next lowest concentration. • Variations in test substance concentrations between replicates of each dose level not reported. • No mortality was observed at any treatment level; the highest nominal concentration tested approximated the functional water solubility of the test substance. (p. 9) • Mean measured concentrations ranged from 89 to 100% of nominal. (pp. 17 and 22)
Concentration Analysis • Performed at test initiation and every 48 hrs • Static: each replicate, minimally at test initiation (before organisms added), at 48 hrs and at end of test • Static-renewal: each replicate, at test initiation and end, and just before and after each renewal • Flow-through: each replicate at 0, 48, and 96 hrs, and every 96 hrs thereafter	• Samples from each test solution and control were analyzed for test substance concentration at test initiation and test termination. (p. 15) • Test substance concentrations were not measured in each individual replicate. At test initiation, samples were taken from the intermediate mixing vessel prior to division into replicate vessels. At test termination, samples were removed from a composite of replicates A and B. (p. 15) • No concentration analysis conducted at 48 hours since preliminary testing indicated that test substance was stable in marine matrix for over 96 hours. (p. 15)

Guideline Criteria	Reported Information
Controls ▪ Consist of same dilution water, conditions, procedures and test population ▪ Negative and/or solvent ▪ Maximum allowable mortality 10% (or 1 mortality if 7 to 10 fish used) for 96 hr period; 10% additional past 96 hrs.	• Control used same dilution water, conditions and procedures as test solutions. (p. 14) • Solvent control (acetone at a concentration of 0.1 mL/L). (p. 14) • No mortality in controls. (pp. 18 and 23)
Replicates ▪ Two per test concentration ▪ Equal volume test solution and number test fish	• 2 replicates per treatment level and control groups. (p. 13) • Each replicate had same volume of test solution and number of fish. (p. 14)
Test Organisms ▪ Minimum 7/replicate (10 preferred) ▪ Equal number per test chamber ▪ Not fed during treatment period ▪ Randomly or impartially assigned to test vessels within 30 min of addition of test substance ▪ Biological observations made at 6 hrs and every 24 hours	• Ten fish per replicate. (p. 14) • Fish not fed during exposure period. (p. 11) • Impartially placed two at a time in each replicate test aquarium; time not specified. (p. 14) • All aquaria examined at 0, 6, 24, 48, 72 and 96 hours of exposure. (p. 14)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in the report?	• Yes. (pp.3-4)
Name of test facilities, test dates and personnel reported?	• Yes. (pp. 5 and 8)
Identification of test substance (including physicochemical characteristics) and purity provided?	• Physicochemical characteristics of test substance not reported. Identification and purity of test substance provided. (p. 11)
Methods used in preparation of stock solutions and analysis of test concentrations described? Accuracy of method (i.e., detection limit and quantification limit) reported?	• Yes. (pp. 13-14 and 40)
LC_{50} concentration-response curves, LC_{50} values, and associated 95% C.I. determined for 24, 48, 72, and 96 hrs? NOEC also reported?	• Concentration-response curves not provided. LC50 values and 95% C.I. for 24, 48, 72 and 96 hours, and NOEC reported. (p. 24)

Guideline Criteria	Reported Information
Graph of concentration-mortality curve at test termination and any control mortality observed during acclimation or study period provided?	• Not applicable; no mortality was observed during the exposure or acclimation period.
Any protocol deviations which may have influenced final results of test reported?	• No.
Raw data included?	• No.
Signs of abnormal behavior by test fish (if any) described?	• No adverse effects were observed. (p. 18)
Statistical methods reported?	• Not applicable. Since there was no mortality or adverse effects, the LC50 values were empirically estimated to be greater than the highest mean measured concentration tested. (p. 16)

Dose Response

Mortality

Nominal Concentration (mg a.i./L)	Mean Measured Concentration (mg a.i./L)	Number of Fish at Test Initiation	Number of Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Control	Control	20	0	0	0	0
Solvent Control	Solvent Control	20	0	0	0	0
6.3	6.1	20	0	0	0	0
13	13	20	0	0	0	0
25	25	20	0	0	0	0
50	49	20	0	0	0	0
100	89	20	0	0	0	0

Statistical Results

Statistical Method: The mean measured concentrations tested and the corresponding mortality data were used to estimate LC_{50} and 95% confidence intervals (CIs). Since no mortalities or adverse effects occurred during the study, the LC_{50} values were empirically estimated to be greater than 89 mg a.i./L, the highest concentration tested. The NOEC was determined, by visual inspection, to be 89 mg a.i./L.

Results Synopsis:

Duration	LC_{50} (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr	> 89	NA[a]	NA
48-hr	> 89	NA	NA
72-hr	> 89	NA	NA
96-hr	> 89	NA	NA
NOEC through 96 hours = 89 mg a.i./L			

a NA = Not applicable; 95% confidence intervals could not be calculated.

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Since no mortality occurred at any of the dose levels tested, LC_{50} values can only be described as being greater than the highest concentration tested, 89 mg a.i./L.

Results Verification Synopsis: 96-hr LC_{50} > 89 mg a.i./L 95% C.I.: Not determined
NOEC: 89 mg a.i./L

14. REVIEWER'S COMMENTS:

There was no mortality at any of test concentrations used in the study. The study authors noted that an attempt to determine an LC_{50} by increasing dose levels was not made, since the highest treatment level approximated the water solubility limit of the test substance.

The pH of the test solutions ranged from 7.1 to 7.9, falling outside the guideline-stipulated range of > 7.5 and < 8.5 for marine testing. Since there were no mortalities or adverse effects during the study, the study authors concluded that this deviation did not have a negative impact on test results.

DATA EVALUATION RECORD
AVIAN DIETARY TOXICITY TEST
GUIDELINE OPPTS 850.2200

1. **CHEMICAL:** 5-(4-chlorophenyl)-4-cyano-1H-pyrrole-2-carboxylic acid **PC Code No.: 119093**

2. **TEST MATERIAL:** R107894, aka CL322248 **Purity:** 94.5 %
CAS No. 122454-29-9
Batch No. 1547-24

3. **CITATION**

Authors:	Sean P. Gallagher, Kathy H. Martin, Joann B. Beavers
Title:	CL322248: A Dietary LC_{50} Study with the Mallard
Study Completion Date:	January 5, 2006
Laboratory:	Wildlife International, Ltd. 8598 Commerce Dr. Easton, Maryland 21601
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse Belgium
Laboratory Report ID:	Janssen Study No.: AGR 1174 Wildlife International, Ltd. Project No.: 168-103
MRID No.:	467513-10

4. **REVIEWED BY:** Kathryn Montague, M.S., Biologist RASSB/AD/US EPA
Signature: Kathryn V. Montague **Date:** 09/19/06

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader RASSB/AD/US EPA
Signature: Siroos Mostaghimi **Date:** 9/20/06

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Anas platyrhynchos*
Age of Test Organism: 10 days
Definitive Test Duration: 5-days exposure, 3-days post-exposure
Study Method: Dietary
Type of Concentrations: Nominal

7. **CONCLUSIONS**

Verified Results Synopsis:
Dietary LC_{50}: >5620 ppm a.i.
No mortality concentration: 5620 ppm a.i.
NOEC: 3160 ppm a.i. (reduction body weight gain)

8. ADEQUACY OF THE STUDY

A. Classification: Acceptable

B. Rationale: No significant deviations from Guideline recommendations

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.2200:

- The OPPTS Guideline states that the relative humidity of the test room should be maintained at 45 to 70%. During this study, the average relative humidity was 36 ± 5%.
- The photoperiod was 16-hr light/8-hr dark during acclimation and throughout the test. The guideline recommends a photoperiod of 14-hr light/10-hr dark.
- It is not known if the avian diet was tested for contaminants periodically throughout the test.
- Spacing of test concentration was not at least 60% of the next highest dose. Nominal test concentrations were slightly lower at 56%. In addition, no mortalities were observed at any of the test concentrations. The test was conducted using the highest recommended treatment level (5000 ppm).

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ Preferred species: either an upland game bird species, preferably the bobwhite (*Colinus virginianus*) or a wild waterfowl species, preferably the mallard (*Anas platyrhynchos*). ▪ If bobwhite purchased, preferable that purchased as eggs which are hatched and reared in testing facility ▪ During incubation of bobwhite quail, recommended temperature is 39°C and relative humidity is 70% ▪ All birds used in test should be from same source and hatch	▪ Mallard (*Anas platyrhynchos*) (p.10). ▪ All birds from same source and hatch (pp. 10 and 11).
Age at Beginning of Test ▪ Bobwhite quail: 10-14 days old ▪ Mallard duck: 5-10 days old ▪ All treatment and control birds should be same age ±1 day. ▪ Exact age should be reported.	▪ 10 days of age (p.10).
Chicks appeared healthy and did not have excessive mortality before the test? Birds should not be used for test if more than 5% of total test population die during 72 hours preceding test	▪ Yes (p.10).
Acclimation Period ▪ Acclimated to test facilities and diet for a minimum of 7 days	▪ All birds acclimated for 7 days (p.13).

B. Test System

Guideline Criteria	Reported Information
Pens ▪ Should be constructed of galvanized metal, stainless steel, or perfluorocarbon plastics ▪ Wire mesh should be used for floors and external walls ▪ Floor area should be at least 300 cm^2/bird for bobwhite quail and 600 cm^2/bird for mallard duck ▪ Should be kept indoors and heated	▪ External walls, ceilings, and floors constructed of vinyl coated wire grid (p.13). ▪ Floor space 62 x 92 cm (5704 cm^2) per pen; with 5 ducklings per pen (1141 cm^2/bird) (pp. 13 and 14). ▪ Pens indoors and thermostatically controlled (p.14).
Room Temperature ▪ 22-38EC	▪ Average room temperature: 19.0 ± 1.1°C (p.14). ▪ Average pen temperature: 29.4 ± 1.5°C (p.14).

Relative Humidity ▪ 45-70%	▪ Average room relative humidity: 36 ± 5% (p.14).
Photoperiod ▪ Recommended 14 hours light/10 hours dark ▪ Continuous lighting is acceptable	▪ Photoperiod: 16-hr light/8-hr dark (p.14).
Diet ▪ A commercial diet for game birds or duck starter mash should be used ▪ Only clean, unmedicated water should be offered during 96 hours preceding test period ▪ Diets should be analyzed periodically for contaminants ▪ Nutrient analysis and list of ingredients in diet should be included in report ▪ Clean water should be available *ad libitum*; if water pans or bowls used water should be changed at least once a day	▪ Birds fed game bird ration formulated to laboratory's specification (p.11). ▪ Water from public water supply and contained no antibiotic medication during acclimation or test (p.11). ▪ Nutrient analysis and list of ingredients included in report, periodic testing for contaminants not indicated (p.22). ▪ Water and feed available *ad libitum* (p.11).

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test ▪ Should be conducted ▪ Generally, groups of a few birds fed 3 to 5 widely spaced concentrations for 5 days ▪ Concentration series of 5, 50, 500, and 5,000 ppm suggested	▪ Dietary concentrations established based upon results of range-finding test in which showed no mortalities or signs of toxicity at dietary concentration of 1000 ppm a.i. Details of test not provided (p.9).
Test Concentrations ▪ Minimum of 5 concentrations spaced geometrically ▪ Recommended spacing is for each concentration to be at least 60% of next highest dose ▪ At least one concentration should kill more than 50% and at least one concentration should kill less than 50% ▪ Treated diets should be analyzed to confirm proper dietary concentration of test substance--should be conducted at beginning of exposure period with samples from high, middle and low concentrations	▪ Five nominal test concentrations: 0 (control), 562, 1000, 1780, 3160, and 5620 ppm a.i. (p.9). ▪ Concentrations 56% of the next highest dose. ▪ No mortalities observed at any concentration (p.15). ▪ Samples of test diets analyzed to verify concentrations and to confirm stability and homogeneity of test substance. Verification samples collected at time of preparation and on Day 5 (control, 1000, 1780 and 3160 ppm a.i.) (pp. 11 and 12).

Controls ▪ Concurrent control group required ▪ Should be from same hatch as those used in treatments ▪ Kept under same environmental conditions	▪ Concurrent control group of 30 ducklings from same hatch and kept under same environmental conditions (pp. 9, 11, and 14).
Number of Birds per Group ▪ Minimum of 10 per test concentration ▪ Minimum of 20 for negative or carrier controls; 30 or more control birds is preferred	▪ Ten ducklings per treatment group (5 per pen) (p.11). ▪ Thirty ducklings in control (5 per pen) (p.11).
Test Substance ▪ Should be mixed in diet evenly ▪ Should be added without use of diluent; if needed preferred diluent is distilled water or if substance is not water soluble, reagent grade evaporative diluent (e.g., acetone or methylene chloride) ▪ Other possible diluents: corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic ▪ If diluent used, should not comprise more than 2% by weight of treated diet ▪ Diets can be mixed by commercial, mechanical food mixers and may be mixed under a hood ▪ Should be mixed freshly just prior to beginning of test	▪ Test diets prepared by mixing test substance directly into feed with Robot Coupe blixer and Hobart mixer (p.11). ▪ No solvent or carrier used (p.11). ▪ Prepared on day of test initiation for each treatment and control group (p.11).
Test Acceptability ▪ No more than 10% of control birds die ▪ Evidence provided that test concentrations were at least 80% of nominal for first 5 days of test period ▪ Lowest treatment level did not result in compound-related mortality or other observable effects	▪ No mortalities in control birds (p.15). ▪ Test concentrations on Day 5 ranged from 98 to 106% of mean Day 0 concentrations and from 96 to 102% of nominal (p.30). ▪ No mortality or treatment-related effects observed at any test concentration (p.15).
Test Durations ▪ 5 days with treated feed and at least 3 days observation with "clean" feed ▪ If any test birds die during 2nd or 3rd day of postexposure period, test period should be extended until 2 successive mortality-free days and 1 day free of toxic signs occur or until 21 days after beginning of test (whichever comes first)	▪ Five days exposure and 3 days post-exposure (p.13). ▪ No mortalities observed during post-exposure period (p. 18).

Observations ▪ Signs of intoxication, abnormal behavior and mortality should be recorded and reported by dose level and by day ▪ Should be made at a minimum 3x on the first day of exposure ▪ Should be made at least twice during remainder of test period; twice daily observations recommended ▪ Average body weights should be reported at beginning and end of nonnal 3-day postexposure period ▪ Average food consumption should be measured either daily or every other day in controls and pens with second lowest and second highest concentration levels; for other pens should be measured for both the exposure period and the normal 3-day postexposure period	▪ Observations recorded by dose-level and by day (Appendix V). ▪ Observations made 4X on first day of exposure (p.14). ▪ Observations made twice daily throughout test (p.14). ▪ Individual body weights measured on Day 0, Day 5, and test termination (Day 8) (p.14). ▪ Average feed consumption determined by pen for each treatment and control group daily during exposure period (Days 0-5) and post-exposure period (p.14).

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	▪ Yes (pp. 3 and 4).
Name of test, sponsor, test laboratory and location, principal investigators and actual dates of beginning and end of test reported?	▪ Yes (pp. 1 and 8).
Name of test species, age, average body weights and individual body weights of all birds that die during test reported?	▪ Yes for all birds (pp. 19 and Appendix VI).
Description of housing conditions (type, size and material of pen, temperatures, humidity, photoperiod and lighting intensity) reported?	▪ Yes (pp. 13 and 14).
Detailed description of diet (source, diluents, supplements, if used) reported? Nutrient analysis of diet included?	▪ Yes (pp. 11, 22, and 23).
Detailed description of test substance including chemical name, source, composition, physical/chemical properties reported?	▪ Yes, with the exception of physical/chemical properties (pp. 10 and 21).
Number of concentrations used, nominal and measured concentrations, number of birds per concentration and for controls reported?	▪ Yes (pp. 9 and 30).
Acclimation procedures reported?	▪ Yes (p. 9).
Frequency, duration and methods of observation reported?	▪ Yes (pp. 14 and Appendix V).
Signs of toxicity (if any) were described?	▪ Yes (p. 15).
Raw data included?	▪ Yes (Appendix V, VI, VII).

Dose Response There were no mortalities in the control and no mortalities or overt signs of toxicity in any of the treatment groups. All birds were normal in appearance and behavior throughout the duration of the test. There was a treatment-related reduction in mean body weight gain at the 5620 ppm a.i. test concentration during the exposure period (Day 0-5). There was no apparent treatment-related effect on food consumption at any concentration tested.

Mortality

Conc. (ppm a.i.)		No. of Birds	Cumulative Number of Dead							
Nominal	Mean Measured		Day of Study							
			1	2	3	4	5	6	7	8
Control (0)	<20	30	0	0	0	0	0	0	0	0
562	542	10	0	0	0	0	0	0	0	0
1000	967	10	0	0	0	0	0	0	0	0
1780	1710	10	0	0	0	0	0	0	0	0
3160	3140	10	0	0	0	0	0	0	0	0
5620	5730	10	0	0	0	0	0	0	0	0

* Day 5

Body Weights

Nominal Concentration (ppm a.i.)	Mean Body Weights (SD) (g)					
	Day of Study					
	Exposure Period			Post Exposure Period		Total Change*
	0	Change*	5	Change*	8	
0	138 (15)	139 (16)	277 (27)	114 (17)	391 (36)	253 (28)
562	139 (16)	128 (13)	266 (21)	103 (11)	369 (25)	230 (19)
1000	138 (16)	141 (17)	279 (30)	108 (6)	387 (33)	249 (20)
1780	138 (15)	136 (14)	274 (22)	106 (13)	379 (28)	241 (22)
3160	139 (17)	124 (19)	262 (29)	126 (13)	388 (37)	250 (26)
5620	138 (16)	99 (18)	237 (27)	123 (13)	360 (29)	222 (21)

*Study Report stated that mean change was calculated separately from the mean body weights using individual body weights provided in the Appendix to the report.

Statistical Results

Statistical Method: The Study Report states that no statistical analyses were applied to the mortality data since no mortalities occurred. In addition, no statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

Results Synopsis:

Dietary LC_{50}: >5620 ppm a.i.
No mortality concentration: 5620 ppm a.i.
NOEC: 3160 ppm a.i. (reduction body weight gain)

I3. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: No statistical analyses were applied to the mortality data since no mortalities occurred. Hence, the LC_{50} value was greater than the highest test concentration. Body weight data were analyzed to determine if there were any statistically significant treatment effects using TOXSTAT. Individual body weights were first checked for normality using the Chi-square Test and for homogeneity of variances using Bartlett's Test. The data passed for both normality and homogeneity of variance. The NOEC was then determined using ANOVA with Bonferroni's Test and William's Test. Body weight gain was significantly reduced at 562 and 5620 ppm, according to Bonferroni's Test; however, the more sensitive William's Test only showed a significant reduction at 5620 ppm.

Results Verification Synopsis:

Dietary LC_{50}: >5620 ppm a.i.
No mortality concentration: 5620 ppm a.i.
NOEC: 3160 ppm a.i. (reduction body weight gain)

I4. REVIEWER'S COMMENTS:

Guideline deviations are listed in Section 9 of this DER.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

November 9, 2006

MEMORANDUM

Subject: Review of the registrant's responses (MRID#s – 469179-01, 469179-02, and 469179-03) in order to upgrade six studies previously reviewed by RSSAB: MRID#s: 465960-01, -04 -06, -11, -12, and -14). These studies are to be used to support the registration of Econea Technical. (DP Barcode: 332684; Decision#: 220066; PC Code: 119093)

From: David C. Bays, Risk Assessment and Science Support Branch (RSSAB), Antimicrobials Division (7510W) 11/7/06

To: Mr. Marshall Swindell, PM-33, Antimicrobials Division (7510W)

Thru: Norm Cook, Branch Chief, RASSB, AD

RSSAB has completed a review of additional information submitted to the Agency in response to six previously reviewed toxicity studies that had been classified as either supplemental or invalid. These studies are listed in the following Table:

CHEMICAL - STUDY TYPE (MRID #)	ORIGINAL CLASSIFICATION	UPGRADED CLASSIFICATION
R107894 - Acute toxicity to water fleas, *Daphnia magna* (MRID#: 465960-01)	Supplemental	Core
R107894 – Full Life Cycle Toxicity Test with water fleas *Daphnia magna* (MRID#: 465960-04)	Invalid	Core
R107894 - Acute Toxicity Test to the Freshwater Green Algae, *Pseudokirchniella subcapitata*	Supplemental	Core

(MRID#: 465960-06		
CL322,250 – Full Life Cycle Toxicity Test with Water fleas, *Daphnia magna* (MRID#: 465960-11)	Supplemental	Study currently under review
CL322,250- Life Cycle Toxicity Test with Mysids, *Americamysis bahia* (MRID#: 465960-12)	Invalid	Study currently under review
CL322,250 – Acute Toxicity to the Marine Diatom, *Skeletonema costatum* (MRID#: 465960-14)	Supplemental	Core

All six studies (See above Table) were upgraded to core based on information provided by the testing laboratory, Springborn Smithers. This information adequately addressed all of the significant guideline deviations that caused each study to be originally classified as either supplemental or invalid. The information addressing each significant guideline deviation has been summarized in the new Core DERs. The complete response by the testing lab is included in MRID# - 469179-01. The raw data on reproduction that addresses a key guideline deviation for MRID# - 465960-11 is found in MRID# - 469179-02. The raw data to verify reproduction and growth endpoints for MRID# - 465960-12 is found in MRID# - 469179-03.

Any questions or comments on this memo should be referred to David Bays at 605-0216.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND

TOXIC SUBSTANCES

December 18, 2006

MEMORANDUM

Subject: Review of the registrant's responses (MRID#s – 469179-01, 469179-02, and 469179-03) in order to upgrade two studies previously reviewed by RSSAB: MRID#s: 465960 -11, and -12). These studies are to be used to support the registration of Econea Technical. (DP Barcode: 332684; Decision#: 220066; PC Code: 119093)

From: David C. Bays, Risk Assessment and Science Support Branch (RSSAB), Antimicrobials Division (7510W) 12/18/06

To: Mr. Marshall Swindell, PM-33, Antimicrobials Division (7510W)

Thru: Norm Cook, Branch Chief, RASSB, AD

RSSAB has completed a review of additional information submitted to the Agency in response to two previously reviewed toxicity studies that had been classified as either supplemental or invalid. These studies are listed in the following Table:

CHEMICAL - STUDY TYPE (MRID #)	ORIGINAL CLASSIFICATION	UPGRADED CLASSIFICATION
CL322,250 – Full Life Cycle Toxicity Test with Water fleas, *Daphnia magna* (MRID#: 465960-11)	Supplemental	Core
CL322,250- Life Cycle Toxicity Test with Mysids, *Americamysis bahia* (MRID#: 465960-12)	Invalid	Core

The two studies (See above Table) were upgraded to core based on information provided by the testing laboratory, Springborn Smithers. This information adequately addressed all of the significant guideline deviations that caused each study to be originally classified as either supplemental or invalid. The information addressing each significant guideline deviation has

been summarized in the new Core DERs. The complete response by the testing lab is included in MRID# - 469179-01. The raw data on reproduction that addresses a key guideline deviation for MRID# - 465960-11 is found in MRID# - 469179-02. The raw data to verify reproduction and growth endpoints for MRID# - 465960-12 is found in MRID# - 469179-03.

Any questions or comments on this memo should be referred to David Bays at 605-0216.

FINAL
5/25/06

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MARCH 30, 2006

MEMORANDUM

Subject: Review of three acute aquatic toxicity studies, using *Oncorhynchus mykiss, Lepomis macrochirus, and Daphnia magna* as test organisms, submitted to support the proposed registration of CL 322,250 a major degradate of Econea Technical. (DP Barcode 327256; Decision# 220066; PC Code 119093)

From: David C. Bays, Risk Assessment and Science Support Branch (RASSB), Antimicrobials Division (7510W) 4/24/06

To: Marshall Swindell, Product Manager #33, Antimicrobials Division (7510W)

Thru: Norm Cook, Branch Chief, RASSB, AD

RASSB has completed the review of three aquatic toxicity studies (MRIDs 46596008, 46596009 and 46596010) with CL 322,250 a major degradate of Econea Technical as the test chemical. Econea Technical is used as an anti-foulant paint product. The first study was an acute aquatic invertebrate acute toxicity test using Freshwater Daphnids, *Daphnia magna*, as the test organism (OPPTS 850.1010). There were some guideline deviations identified by the reviewer, but these were minor in nature and did not affect the results of the study (see DER for MRID 46596008). Therefore, the study is classified as core and can be used in a risk assessment. As reported, the results were as follows: 48-hour EC_{50} was 0.51 mg a.i./L (95% C.I. = 0.42-0.61 mg a.i./L) and the NOEC was 0.25 mg a.i./L, which indicates that CL 322,250 is acutely highly toxic to freshwater daphnids.

The second study (MRID 46596009) was a fish acute toxicity test using Bluegill Sunfish, *Lepomis macrochirus*, as the test organism (OPPTS 850.1075). There were some guideline deviations identified by the reviewer, but these were minor in nature and did not affect the results of the study (see DER for MRID 46596009). Therefore, the study is classified as core and its results can be used in a risk assessment. As reported, the results were as follows: 96-hour LC50 was 1.2 mg a.i./L (95% C.I. = 1.1-1.4 mg a.i./L) and the 96-hour NOEC was 0.55 mg a.i./L, which indicates that CL 322,250 is acutely moderately toxic to bluegill sunfish.

The third study (MRID 46596010) was a fish acute toxicity test using Rainbow Trout, *Oncorhnchus mykiss*, as the test organism (OPPTS 850.1735). There were some guideline deviations identified by the reviewer, but these were minor in nature and did not affect the results of the study (see DER for MRID 46596010). Therefore, the study is classified as core and can be used in a risk assessment. As reported, the results were as follows: 96-hour LC50 was 520 µg a.i./L (95% C.I. = 320-870 µg a.i./L) and the NOEC was 320 µg a.i./L, which indicates that CL 322,250 is acutely highly toxic to rainbow trout.

If you have any questions on the above, please contact David Bays at 703-605-0216.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

EPA United States Environmental Protection Agency Office of Pesticide Programs

April 24, 2007

MEMORANDUM

SUBJECT: Econea (tralopryil) Antifoulant New Chemical Registration, Ecological Hazard Assessment and Environmental Risk Characterization (PC Code: 119093)

FROM: Richard C. Petrie, Senior Agronomist/Team Leader R.C. 4/24/07
Kathryn Montague, Biologist
Siroos Mostaghimi, Environmental Engineer/Team Leader. Siroos Mostaghimi
James Breithaupt, Agronomist/Environmental Fate Scientist
Risk Assessment and Science Support Branch
Antimicrobials Division (7510P)

THRU: Norm Cook, Branch Chief 4/24/07
Risk Assessment and Science Support Branch
Antimicrobials Division (7510P)

TO: **Marshall Swindell, RM 33**
Antimicrobials Division (7510P)

Attached is the ecological hazard and environmental risk characterization for the use of Econea (tralopryil) as an antifoulant. If you have any questions, please contact Richard Petrie (703-305-7358) or Norm Cook (703-308-8253).

Table of Contents

ECONEA™ (119093)

Ecological Hazard Assessment and Environmental Risk Characterization

Executive Summary:

ECONEA™ [1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)], also known as tralopryil, AC303268, R107894, or AF028 is a copper free antifoulant. Econea will be mixed with paint in a slow-release matrix to control hard fouling organisms such as barnacles, mussels, and polychaetes found on the hulls of boats and vessels, as well as on marine structures.

Econea (tralopryil) is rapidly hydrolyzed once released from the paint matrix into water forming the primary degradate 322,250. Both tralopryil and the primary degradate 322,250 are quickly adsorbed to suspended solids and sediments in surface water making them mostly unavailable to fish and aquatic invertebrates within the water column. Tralopryil has a longer ½ life in fresh water than marine water (3-177 days depending on pH vs 0.6 days respectively), however, it has a higher adsorption coefficient to suspended solids and sediments in fresh water than in marine water (Koc = 20,273 vs 5229 respectively). Tralopryil is not likely to bioaccumulate in freshwater and saltwater fish based on bioconcentration estimates. A second degradate CL322,248, a debrominated form of CL322,250, is formed in fresh and salt water and persists in water and sediment longer than parent tralopryil. A third minor degradate, CL325,195 is a debrominated form from 322,250. CL325,195 is not formed in freshwater and is expected to occur in very low concentrations in saltwater.

This risk assessment will focus on the registration of Econea for use on large ships only. Large ocean-going vessels are expected to spend the majority of their useful lives in open waters. Econea treated ships will traverse fresh and marine waters within and around the U.S. Tralopryil concentrations around treated ships are expected to pose a negligible risk in saltwater and in most freshwater areas due to environmental fate factors that lead to reduced residues in surface waters, plus low bioconcentration potential. The risk will further diminish as a treated ship moves through open water because of immediate dilution of parent tralopryil in large water bodies. While docked, tralopryil is expected to quickly hydrolyze to degradates and adsorb to sediments in water. In freshwater, hydrolysis occurs more slowly therefore tralopryil may reach acutely toxic levels to aquatic organisms in the immediate vicinity of the ship for a short period of time if little or no tidal flushing occurs if docked for several days. Overall risk to aquatic organisms while a ship is docked is not expected to be a significant concern because large ships remain in areas where sediments in the water column are high, ship movements create wave washing, and loading/unloading occurs quickly. For these reasons, the focus of this environmental risk assessment is on the primary and secondary degradates of tralopryil that are expected to occur in the environment for a longer period of time that parent tralopryil.

A large number of ecotoxicity studies for tralopryil and it's degradates were submitted. This deterministic risk assessment used the maximum expected environmental concentration

(EEC) and toxicity endpoints for avian species, fish, invertebrates, estuarine/marine species, sediment dwelling species, and plants to generate risk quotients for the degradates 322,250 and 322,248. Some limited ecotoxicity studies were submitted for the minor degradate 325,195 however it is not a concern due to its relatively low rate of formation and low toxicity to test species. Terrestrial bird, mammal, or plant exposure is not expected to occur to any great extent for antifoulants because the treated paint stays at or below the water line. However, risks to waterfowl exposed to tralopryil and degradates through feeding were assessed based on avian concerns when compounds similar to tralopryil are used in agricultural fields.

Three different aquatic models were used to predict expected environmental concentrations (EEC's) in harbors, marinas, bays, and channels in U.S. waters. Model runs submitted were reviewed for completeness and accuracy by the Agency. MAM-PEC (Marine Antifouling Model to Predict Environmental Concentrations) Model results, which had the highest EECs (Barbours Point marina, Houston) were used in this risk assessment.

Parent Econea (tralopryil) is acutely toxic to birds and aquatic species but its use as an antifoulant paint on large ships is not expected to pose direct acute or chronic risk to non-target avian species, mammalian species, aquatic animals, sediment dwellers, or plants in fresh or salt water. The only area having potential for very limited acute toxicity to aquatic organisms is a fresh/clear water dockage having little or no water exchange around a ship that is docked for a long period of time. Econea areas of exposure may overlap with listed species which warrants a more refined assessment, to include indirect and habitat effects. A more refined assessment involves clear delineation of the action area associated with proposed use of Econea antifoulant and use of best available information on the temporal and spatial co-location of listed species with respect to the action area. Because refined risk assessment has not been conducted for this action an endangered species effect determination will not be made at this time.

Outstanding data include:

Degradate CL322,250

850.1075/72-1 Freshwater fish acute toxicity testing with coldwater species (Rainbow trout)

1. ECOLOGICAL HAZARD ASSESSMENT

A. Toxicity to Terrestrial Animals

1. Birds, Acute and Subacute

An acute oral toxicity study using the technical grade of the active ingredient is required to establish the toxicity of a pesticide to birds. This information is used to determine label hazard statements, as well as to estimate risk for pesticides which could be directly ingested by birds. The preferred test species is either mallard duck or bobwhite quail. Results of this test are tabulated below.

Table 1. Avian Acute Oral Toxicity of tralopryil (parent)

Species	% a.i.	LD50 (mg ai/kg) 95 % c.i.	NOAEL	Toxicity category	MRID No. Author/Year	Study Classification
Mallard (*Anas platyrhynchos*)	100.3	77 (57 – 104), slope 5.47	40 (survival) ; 20 (weight)	Moderately toxic	434928-08 Campbell et al., 1994	Acceptable
Northern bobwhite (*Colinus virginianus*)	100.3	24.7 (17.3 – 35.3), slope 3.4	6 (survival and weight)	Highly toxic	434928-09 Campbell et al., 1994	Acceptable

These results indicate that tralopryil technical is moderately to highly toxic to avian species on an acute oral basis. Guideline 850.2100/72-1 is fulfilled.

Avian acute oral testing was also conducted on the CL325,195 metabolite of tralopryil. The results of this testing are provided in the table, below

Table 2. Avian Acute Oral Toxicity of CL 325,195						
Species	**% a.i.**	**LD50 (mg ai/kg) 95 % c.i.**	**NOAEL**	**Toxicity category**	**MRID No. Author/Year**	**Study Classification**
Mallard (*Anas platyrhynchos*)	97	>2250	2250	Practically non-toxic	444526-12 Gagne et al., 1997	Acceptable
Northern bobwhite (*Colinus virginianus*)	97	741 (549 – 3017); slope 4.17	192 (reduced feed consump-tion)	Slightly toxic	444526-11 Gagne et al., 1997	Acceptable

These results indicate that the CL325,195 degradate is slightly toxic to practically non-toxic to birds on an acute oral basis.

Two subacute dietary studies using the technical grade of the active ingredient are required to establish the toxicity of a pesticide to birds, if the use of the pesticide is expected to result in exposure to birds via food items. The preferred test species are mallard duck (a waterfowl) and bobwhite quail (an upland gamebird). Avian dietary testing with waterfowl was required for Econea™ technical and the two major aquatic degradates in order to address the toxicity of the compounds to waterfowl, which may be exposed through feeding in waters containing Econea™ or its degradates. Results of the submitted avian subacute dietary tests are provided in the tables, below.

Table 3. Avian Subacute Dietary Toxicity of tralopryil Technical						
Species	**% ai**	**LC50 (ppm) (95 % c.i.)**	**NOAEC (ppm)**	**Toxicity Category**	**MRID No. Author/Year**	**Study Classification**
Mallard *(Anas platyrhynchos)*	94.6	10.76 (5.62 – 17.8)	10 (growth) No mortality level 5.62	Very Highly Toxic	465960-05 Gallagher et al., 2005	Acceptable

These results indicate that tralopryil is very highly toxic to waterfowl when ingested via food items.

Table 4. Avian Subacute Dietary Toxicity of CL322,250

Species	% ai	LC50 (ppm) (95 % c.i.)	NOAEC (ppm)	Toxicity Category	MRID No. Author/ Year	Study Classification
Mallard *(Anas platyrhynchos)*	88.2	962 (716 – 1300)	250 (toxicity) No mortality level 500	Highly toxic	465960-13 Gallagher et al., 2005	Acceptable

These results indicate that CL322,250 is highly toxic to waterfowl when ingested via food items.

Table 5. Avian Subacute Dietary Toxicity of CL322,248

Species	% ai	LC50 (ppm) (95 % c.i.)	NOAEC (ppm)	Toxicity Category	MRID No. Author /Year	Study Classification
Mallard *(Anas platyrhynchos)*	94.5	>5620	3160	Practically non-toxic	467513-10	Acceptable

These results indicate that CL322,248 is practically non-toxic to waterfowl when ingested via food items.
The avian dietary testing Guideline (850.2200/71-2) is fulfilled.

2. Birds, Chronic

Avian reproduction studies using the technical grade of the active ingredient are required for a pesticide when any of the following conditions are met: (1) birds may be subject to repeated or continuous exposure to the pesticide, especially preceding or during the breeding season, (2) the

pesticide is stable in the environment to the extent that potentially toxic amounts may persist in animal feed, (3) the pesticide is stored or accumulated in plant or animal tissues, and/or, (4) information derived from mammalian reproduction studies indicates reproduction in terrestrial vertebrates may be adversely affected by the anticipated use of the product. Avian reproduction testing is not required for the currently proposed uses of Econea™.

3. Mammals, Acute and Chronic

Wild mammal testing is required on a case-by-case basis, depending on the results of lower tier laboratory mammalian studies, intended use pattern and pertinent environmental fate characteristics. In most cases, rat or mouse toxicity values obtained from studies conducted to support data requirements for human health risk assessment substitute for wild mammal testing. These toxicity values are reported in the table below.

Table 6: Summary of Mammalian Toxicology Endpoints (Excerpted from Toxicity Document Supporting this Registration)

Guideline	Species	Results	Reference	Classification
870.1100 Acute oral (limit test)	Rat	LD50 not determined; Tox category I	MRID 456739-15	Acceptable
870.1200 Acute dermal	Rabbit	LD50 > 2000 mg/kg for both males and females; Tox category III	MRID 456739-16	Acceptable
870.2500 Dermal irritation (limit test)	Rabbit	Mildly irritating based on very slight erythema, but no edema at 72 hours; Tox category IV	MRID 456739-18	Acceptable
870.2400 Primary eye irritation (limit test)	Rabbit	Mildly irritating; Tox category III	MRID 465394-01	Acceptable
870.2600 Dermal sensitization	Guinea pig	Not a sensitizer	MRID 456739-19	Acceptable

870.3700 Developmental Toxicity	Rat	Maternal LOAEL = 10 mg/kg/day based on frequent salivation; Maternal NOAEL = 5 mg/kg/day Developmental LOAEL = 10 mg/kg/day, based on decreased fetal weight; Developmental NOAEL = 5 mg/kg/day	MRID 464269-02	Acceptable
870.3200 Subchronic (28 day) Dermal (range-finding study)	Rat	NOAEL ≤ 1000 mg/kg/day LOAEL > 1000 mg/kg/day	MRID 466597-02	Acceptable
870.3100 Subchronic (90 day) Oral	Rat	Males: NOAEL = 5.2 mg/kg/day, LOAEL = 16.2 mg/kg/day, based on reduced body weight and body weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord. Females: NOAEL = could not be determined LOAEL = 6.3 mg/kg/day (LDT) in females based microscopic findings of the brain and spinal cord.	MRID 466597-01	Acceptable
870.3250 Subchronic (90 day) Dermal	Rat	Dermal: NOAEL = 100 mg/kg/day LOAEL = 300 mg/kg/day, based on appearance of sores Systemic: NOAEL = 300 mg/kg/day, LOAEL = 1000 mg/kg/day based on histopathology changes in the lungs	MRID 468022-01	Acceptable

4. Insects

Nontarget insect toxicity testing is not required for tralopryil or its degradates.

5. Terrestrial Field Testing

Terrestrial field testing is not required for tralopryil or its degradates.

B. Toxicity to Freshwater Aquatic Animals

1. Freshwater Fish, Acute

Two freshwater fish toxicity studies using the technical grade of the active ingredient are required to establish the toxicity of a pesticide to fish. This information is used to determine label hazard statements, as well as to estimate risk to freshwater fish from the proposed use. The preferred test species are rainbow trout (a coldwater fish) and bluegill sunfish (a warmwater fish). Results of these tests are tabulated below.

Table 7. Freshwater Fish Acute Toxicity of tralopryil (parent)

Species	% ai	LC50 (ppb ai) (95 % c.i.)	NOAEC (ppb ai)	Toxicity Category	MRID No. Author/Year	Study Classification
Rainbow trout *(Oncorhynchus mykiss)*	94.6	Flow-through 96-hr LC50 = 1.3 (0.68 – 2.1)	0.68	Very highly toxic	465960-02 Putt, 2005	Acceptable
Bluegill sunfish *(Lepomis macrochirus)*	94.6	Flow-through 96-hr LC50 = 3.2 (2.8 – 3.7)	1.3	Very highly toxic	465960-03 Putt, 2005	Acceptable

These results indicate that tralopryil is very highly toxic to freshwater fish on an acute basis. The guideline requirement (72-1/OPPTS 850.1075) is fulfilled.

Freshwater fish acute toxicity tests with several tralopryil degradates were submitted, however, those studies were determined to be invalid and are therefore not included in this assessment. Based on the patterns of toxicity seen in marine/estuarine fish acute tests, and in freshwater fish chronic tests, the degradates of concern are less toxic than parent tralopryil. Repeat freshwater fish acute testing with the degradates is not required.

2. Freshwater Fish, Chronic

A freshwater fish early life-stage test using the technical grade of the active ingredient is required for a pesticide when it may be applied directly to water or if the end-use product is expected to be transported to water from the intended use site, and any of the following conditions are met: (1) the pesticide is intended for use such that its presence in water is likely to be continuous or recurrent regardless of toxicity, (2) any aquatic acute LC50 or EC50 is less than 1 mg/l, (3) the EEC in water is equal to or greater than 0.01 of any acute LC50 or EC50 value, or, (4) the actual or estimated environmental concentration in water resulting from use is less than 0.01 of any acute LC50 or EC50 value and any one of the following conditions exist: studies of other organisms indicate the reproductive physiology of fish may be affected, physicochemical properties indicate cumulative effects, or the pesticide is persistent in water (e.g., half-life greater than 4 days). The preferred test species is rainbow trout, but other species may be used.. Freshwater fish early life-stage testing was required for tralopryil and its major aquatic degradate, CL322,250, due to the very high acute toxicity of parent tralopryil to freshwater fish and the rapid breakdown of parent tralopryil into CL322,250. The results of this testing is summarized below:

Table 8. Freshwater Fish Early Life-Stage Toxicity of tralopryil (parent)

Species	% ai	NOAEC/ LOAEC (ppb)	Endpoints Affected	MRID No. Author/Year	Study Classification
Zebra fish *(Danio rario)*	94.6	NOAEC: 0.17 LOEC: 0.37	Larval wet and dry weight	458939-01 Sousa, 2003	Acceptable

Table 9. Freshwater Fish Early Life-Stage Toxicity of CL322,250					
Species	**% ai**	**NOAEC/ LOAEC (ppb)**	**Endpoints Affected**	**MRID No. Author/Year**	**Study Classification**
Zebra fish (*Danio rario*)	88.2	NOAEC: 69 LOEC: 140	Larval survival	467503-07	Acceptable
		NOAEC: 270 LOEC: 530	Hatchling survival		
		NOAEC: > 530 LOEC: 530	Growth (length and wet weight)		

3. Freshwater Invertebrates, Acute

A freshwater aquatic invertebrate toxicity test using the technical grade of the active ingredient is required to establish the toxicity of a pesticide to invertebrates. The preferred test species is *Daphnia magna*. Results of this test are tabulated below.

Table 10. Freshwater Invertebrate Acute Toxicity of tralopryil (parent)						
Species	**% ai**	**LC50 or EC50 (ppb ai) (95% c.i.)**	**NOAEC (ppb ai)**	**Toxicity Category**	**MRID No. Author/ Year**	**Study Classification**
Waterflea *(Daphnia magna)*	94.6	Flow-through 48-hr EC50 = 1.5 (1.2 – 1.9)	0.32	Very highly toxic	465960-01 Cafarella, 2005	Core

The daphnid acute study (MRID #465960-01) was classified as supplemental due to unexplained

low percent recoveries in the measured chemical analysis. This is likely due to rapid degradation of the parent compound to CL322,250. The results indicate that parent tralopryil is very highly toxic to aquatic invertebrates on an acute basis.

Table 11. Freshwater Invertebrate Toxicity of CL 322,250

Species	% ai	LC50 or EC50 (ppb ai)	NOAEC (ppb ai)	Toxicity Category	MRID No. Author/ Year	Study Classification
Waterflea *(Daphnia magna)*	93	Static 1630 (1000-2130)	6250	Moderately toxic	457069-03 Van der Kerken, 2002	Supplemental
Waterflea *(Daphnia magna)*	93	Static 700 (590 – 820)	<600	Highly toxic	456741-02 Van der Kerken, 2001	Supplemental

The results indicate that Cl322,250 is moderately to highly toxic to aquatic invertebrates on an acute basis.

Table 12. Freshwater Invertebrate Acute Toxicity of CL 322,248

Species	% ai	LC50 or EC50 (ppb ai)	NOAEC (ppb ai)	Toxicity Category	MRID No. Author/ Year	Study Classification
Waterflea *(Daphnia magna)*	98	Static 16800 (11800-23800)	11800	Slightly toxic	456741-12 Van der Kerken, 2002	Supplemental

The results indicate that CL322,248 is slightly toxic to aquatic invertebrates on an acute basis.

Table 13. Freshwater Invertebrate Acute Toxicity of CL 325,195						
Species	**% ai**	**LC50 or EC50 (ppb ai)**	**NOAEC (ppb ai)**	**Toxicity Category**	**MRID No. Author/ Year**	**Study Classification**
Waterflea *(Daphnia magna)*	97	3510 (2700 – 4300)	<2700	Moderately toxic	457069-02 Van der Kerken, 2002	Supplemental

The results indicate that CL325,195 is moderately toxic to aquatic invertebrates on an acute basis.

4. Freshwater Invertebrate, Chronic

A freshwater aquatic invertebrate life-cycle test using the technical grade of the active ingredient is required for a pesticide if the end-use product may be applied directly to water or expected to be transported to water from the intended use site, and any of the following conditions are met: (1) the pesticide is intended for use such that its presence in water is likely to be continuous or recurrent regardless of toxicity, (2) any aquatic acute LC50 or EC50 is less than 1 mg/l, or, (3) the EEC in water is equal to or greater than 0.01 of any acute EC50 or LC50 value, or, (4) the actual or estimated environmental concentration in water resulting from use is less than 0.01 of any aquatic acute EC50 or LC50 value and any of the following conditions exist: studies of other organisms indicate the reproductive physiology of invertebrates may be affected, physicochemical properties indicate cumulative effects, or the pesticide is persistent in water (e.g., half-life greater than 4 days). The preferred test species is *Daphnia magna*. Freshwater aquatic invertebrate life-cycle testing was required for Econea due to the likelihood of repeated or continuous exposure from boat hulls, as well as the high acute toxicity to freshwater invertebrates. Results of this test are tabulated below.

Table 14. Freshwater Aquatic Invertebrate Life-Cycle Toxicity of tralopryil (parent)					
Species	**% ai**	**NOAEC/ LOAEC (ppb)**	**Endpoints Affected**	**MRID No. Author/ Year**	**Study Classification**
Waterflea *(Daphnia magna)*	94.6	0.20/0.57	Dry weight and length	465960-04 Cafarella, 2005	Acceptable

Table 15. Freshwater Aquatic Invertebrate Life-Cycle Toxicity of CL322,250					
Species	**% ai**	**NOAEC/ LOAEC (ppb)**	**Endpoints Affected**	**MRID No. Author/ Year**	**Study Classification**
Waterflea *(Daphnia magna)*	92.6	300/540	Reproduction and growth	465960-11 Cafarella, 2005	Acceptable

Table 16. Freshwater Aquatic Invertebrate Life-Cycle Toxicity of CL322,248					
Species	**% ai**	**NOAEC/ LOAEC (ppb)**	**Endpoints Affected**	**MRID No. Author/ Year**	**Study Classification**
Waterflea *(Daphnia magna)*	98	<1370/1370 2700/5480	Length of parent daphnids # of offspring	456741-13 Van der Kerken, 2002	Supplemental

These studies show that the parent compound and degradates can cause growth and reproductive effects in aquatic invertebrates, but the degradates are substantially less toxic than the parent compound.

5. Freshwater Field Studies

Freshwater field testing is not required for tralopryil or its degradates.

C Toxicity to Estuarine and Marine Animals

1. Estuarine and Marine Fish, Acute

Acute toxicity testing with estuarine/marine fish using the technical grade of the active ingredient is required for a chemical when the end-use product is intended for direct application to the marine/estuarine environment or the active ingredient is expected to reach this environment because of its use in coastal counties. The preferred test species is sheepshead minnow. This testing is required for antifoulants. Summaries of the results of studies submitted with Econea and degradates are provided in the tables, below.

Table 17. Acute Toxicity of tralopryil (parent) to Estuarine/Marine Fish						
Species	**% ai**	**LC50 (ppb ai) (95% c.i.)**	**NOAEC (ppb ai)**	**Toxicity Category**	**MRID No. Author/ Year**	**Study Classification**
Sheepshead minnow *(Cyprinodon variegatus)*	94.6	Flow-through 23.71 (19.9 – 29.3)	10.0	Very highly toxic	456740-03 and 467513-06/Lima, 2001, and Hoberg 2006	Acceptable

The results indicate that tralopryil parent is highly toxic to estuarine/marine fish on an acute basis. The guideline requirement (72-3a/OPPTS 850.1025) is fulfilled (MRID #43864605).

Table 18: Acute Toxicity of CL322,250 to Estuarine/Marine Fish						
Species	**% ai**	**LC50 (ppb ai) (95% c.i.)**	**NOAEC (ppb ai)**	**Toxicity Category**	**MRID No. Author/ Year**	**Study Classification**
Sheepshead minnow *(Cyprinodon variegatus)*	93	>950	950	Highly toxic	456741-01 Lima, 2001	supplemental

The results indicate that CL322,250 is highly toxic to marine/estuarine fish on an acute basis.

Table 19. Acute Toxicity of CL322,248 to Estuarine/Marine Fish						
Species	**% ai**	**LC50 (ppb ai) (95% c.i.)**	**NOAEC (ppb ai)**	**Toxicity Category**	**MRID No. Author/ Year**	**Study Classification**
Sheepshead minnow *(Cyprinodon variegatus)*	94.5	Static >89000	89000	Slightly toxic	467513-09 Sayers, 2006	Acceptable

The results indicate that CL322,248 is slightly toxic to estuarine/marine fish on an acute basis.

Table 20. Acute Toxicity of CL 325,195 to Estuarine/Marine Fish						
Species	**% ai**	**LC50 (ppb ai) (95% c.i.)**	**NOAEC (ppb ai)**	**Toxicity Category**	**MRID No. Author/ Year**	**Study Classification**
Sheepshead minnow *(Cyprinodon variegatus)*	96	>16000	16000	Slightly toxic	456740-13 Lima, 2001	supplemental

The results indicate that CL325,195 is slightly toxic to marine/estuarine fish on an acute basis.

2. Estuarine and Marine Fish, Chronic.

Table 21. Estuarine/Marine Fish Early Life-Stage Toxicity of tralopryil (parent)					
Species	**% ai**	**NOAEC/ LOAEC (ppb)**	**Endpoints Affected**	**MRID No. Author/ Year**	**Study Classification**
Sheepshead minnow (*Cyprinodon variegatus*)	94.6	4.3/8.7	Larval wet weight and survival	456740-07 Sousa, 2001	Acceptable

Table 22. Estuarine/Marine Fish Early Life-Stage Toxicity of CL 322, 250					
Species	**% ai**	**NOAEC/ LOAEC (ppb)**	**Endpoints Affected**	**MRID No. Author/ Year**	**Study Classification**
Sheepshead minnow (*Cyprinodon variegatus*)	93 - 94	240/510	Larval survival, length, wet weight, dry weight	456741-06 Sousa, 2001	Acceptable

Table 23. Estuarine/Marine Fish Early Life-Stage Toxicity of CL 325,195					
Species	**% ai**	**NOAEC/ LOAEC (ppb)**	**Endpoints Affected**	**MRID No. Author/ Year**	**Study Classification**
Sheepshead minnow (*Cyprinodon variegatus*)	95	1300/2700	Larval survival	456740-17 Sousa, 2001	Acceptable

These data demonstrate that the parent compound and degradates are capable of impacting fish larval survival and growth. The degradates are substantially less toxic than the parent compound.

3. Estuarine and Marine Invertebrates, Acute

Acute toxicity testing with estuarine/marine invertebrates using the technical grade of the active ingredient is required for a pesticide when the end-use product is intended for direct application to the marine/estuarine environment or the active ingredient is expected to reach this environment because of its use in coastal counties. The preferred test species are mysid shrimp and eastern oyster. Results of these tests are tabulated below.

Table 24: Acute Toxicity of tralopryil (parent) to Estuarine/Marine Invertebrates						
Species	**% ai.**	**96-hour LC50/EC50 (ppb) (95% c.i.)**	**NOAEC (ppb)**	**Toxicity Category**	**MRID No. Author/ Year**	**Study Classification**
Eastern oyster *(Crassostrea virginica)* shell deposition	94.6	Flow through EC50 = 0.64 (0.34 – 1.21)	0.29	Very highly toxic	467513-05 Cafarella, 2006	Acceptable
Eastern oyster *(Crassostrea virginica)* shell deposition	94.6	Flow-through EC50 = 0.56 (0.48-0.65)	<0.19	Very highly toxic	456740-05 Dionne, 2001	Supplemental (lack of NOEC)

Table 24: Acute Toxicity of tralopryil (parent) to Estuarine/Marine Invertebrates						
Species	**% ai.**	**96-hour LC50/EC50 (ppb) (95% c.i.)**	**NOAEC (ppb)**	**Toxicity Category**	**MRID No. Author/ Year**	**Study Classification**
Mysid (*Mysidopsis bahia*)	94.6	Flow-through LC50 = 0.98 (0.83-1.17)	1.5	Very highly toxic	456740-06 Lima, 2001	Acceptable

The results indicate that parent tralopryil is very highly toxic to estuarine/marine invertebrates on an acute basis.

Two freshwater estuarine/marine invertebrate acute toxicity studies using the degradates were submitted.

Table 25. Acute Toxicity of CL 322.250 to Estuarine/Marine Invertebrates						
Species	**% ai.**	**96-hour LC50/EC50 (ppb) (95% c.i.)**	**NOAEC (ppb)**	**Toxicity Category**	**MRID No. Author / Year**	**Study Classification**
Eastern oyster *(Crassostrea virginica)*	94	Flow-through Shell deposition EC50 = 310 (270 – 340)	46	Highly toxic	456741-03 Dionne, 2001	Acceptable
Mysid (*Mysidopsis bahia*)	93	Flow-through 550 (490 – 630)	330	Highly toxic	456741-04 Putt, 2001	Acceptable

The results indicate that CL322,250 is highly toxic to estuarine/marine invertebrates on an acute basis.

Table 26. Acute Toxicity of CL322,248 to Estuarine/Marine Invertebrates						
Species	**% ai.**	**96-hour LC50/EC50 (ppb) (95% c.i.)**	**NOAEC (ppb)**	**Toxicity Category**	**MRID No. Author /Year**	**Study Classification**
Eastern oyster *(Crassostrea virginica)* shell deposition						
Mysid (*Mysidopsis bahia*)	94.5	Static 4300 (3300 – 5400)	2500	Moderately toxic	467513-08 Sayers, 2006	Acceptable

The results indicate that CL322,248 is moderately toxic to estuarine/marine invertebrates on an acute basis.

Table 27. Acute Toxicity of CL 325,195 to Estuarine/Marine Invertebrates						
Species	**% ai.**	**96-hour LC50/EC50 (ppb) (95% c.i.)**	**NOAEC (ppb)**	**Toxicity Category**	**MRID No. Author /Year**	**Study Classification**
Eastern oyster *(Crassostrea virginica)* shell deposition	95	Flow-through >14000	6900	Slightly toxic	456740-14 Dionne, 2001	Acceptable
Mysid (*Mysidopsis bahia*)	96	Flow-through 12250 (10000 – 15000)	5300	Slightly toxic	456740-15 Putt, 2001	Acceptable

The results indicate tha CL325,195 is slightly toxic to marine/estuarine invertebrates on an acute basis.

4. Estuarine and Marine Invertebrate, Chronic

An estuarine/marine invertebrate life-cycle toxicity test is required for a pesticide if the end-use product may be applied directly to water or expected to be transported to water from the intended use site, and any of the following conditions are met: (1) the pesticide is intended for use such that its presence in water is likely to be continuous or recurrent regardless of toxicity, (2) any aquatic acute LC50 or EC50 is less than 1 mg/l, or, (3) the EEC in water is equal to or greater than 0.01 of any acute EC50 or LC50 value, or, (4) the actual or estimated environmental concentration in water resulting from use is less than 0.01 of any aquatic acute EC50 or LC50 value and any of the following conditions exist: studies of other organisms indicate the reproductive physiology of invertebrates may be affected, physicochemical properties indicate cumulative effects, or the pesticide is persistent in water (e.g., half-life greater than 4 days). Estuarine/marine invertebrate life-cycle testing was submitted for tralopryil.

Table 28. Chronic Toxicity of tralopryil (parent) to Estuarine/Marine Invertebrates

Species	% ai.	LOEC (ppb)	NOAEC (ppb)	MRID No. Author/ Year	Study Classification
Mysid (*Mysidopsis bahia*)	98.2	growth: 4.20 ppb repro: 9.16 ppb	growth: 2.28 ppb repro: 4.20 ppb	44911101 Boeri et al/1999	Acceptable
Mysid (*Mysidopsis bahia*)	94.6	Reproductive success: 0.25	Reproductive success: 0.51	456740-09 Sousa, 2001	Supplemental

Table 29. Chronic Toxicity of CL322,250 to Estuarine/Marine Invertebrates

Species	% ai.	LOEC (ppb)	NOAEC (ppb)	MRID No. Author/ Year	Study Classification
Mysid (*Mysidopsis bahia*)	88.2	Repro: 160.0	Repro: 82.0	465960-12 Cafarella, 2005	Acceptable

5. Estuarine and Marine Field Studies

Aquatic field testing is not required for triclopryil or degradates.

D. Sediment Toxicity

Sediment toxicity testing is required in certain cases, based on environmental fate characteristics which indicate the chemical or its metabolites is expected to partition to sediment, and ecological effects information which indicate high toxicity to aquatic invertebrate species. Sediment testing was submitted for tralopryil and its major metabolites, and is summarized in the tables, below.

Table 30. Whole Sediment Toxicity of tralopryil (Parent) to Invertebrates, Freshwater and Marine

Species	% a.i.	10-day LC50 (mg/kg dry sediment) (95% c.i.)	NOAEC/LOAEC, endpoint	MRID Author, Year	Study Classification
Freshwater Amphipod (*Hyalella azteca*)	94.6	2.2 (2.0 – 2.4)	1.0/1.9, survival	456740-10 Cafarella, 2001	Acceptable
Marine Amphipod (*Leptocheirus plumulosa*)	94.6	1.1 (0.95 – 1.2)	0.50/1.1, survival	456740-11 Putt, 2001	Acceptable

Table 31. Whole Sediment Toxicity of CL 322,250 to Invertebrates, Freshwater and Marine					
Species	**% a.i.**	**10-day LC50 (mg/kg dry sediment) (95% c.i.)**	**NOAEC/LOAEC, endpoint**	**MRID Author, Year**	**Study Classification**
Freshwater Amphipod (*Hyalella azteca*)	93	> 35	35/>35	456741-08 Cafarella, 2001	Acceptable
Marine Amphipod (*Leptocheirus plumulosa*)	93	> 70	70/>70	456741-09 Putt, 2001	Acceptable

Table 32. Whole Sediment Toxicity of CL 322,248 to Invertebrates, Freshwater and Marine					
Species	**% a.i.**	**10-day LC50 (mg/kg dry sediment) (95% c.i.)**	**NOAEC/LOAEC, endpoint**	**MRID Author, Year**	**Study Classification**
Marine Amphipod (*Leptocheirus plumulosa*)	98	>75	75/>75	456741-14 Putt, 2001	Acceptable

Table 33. Whole Sediment Toxicity of CL 325,195 to Invertebrates, Freshwater and Marine					
Species	**% a.i.**	**10-day LC50 (mg/kg dry sediment) (95% c.i.)**	**NOAEC/LOAEC, endpoint**	**MRID Author, Year**	**Study Classification**
Freshwater Amphipod (*Hyalella azteca*)	96	> 49	49/>49, survival	456740-19 Cafarella, 2001	Supplemental
Marine Amphipod (*Leptocheirus plumulosa*)	96	>27	27/>27, survival	456740-20 Putt, 2001	Acceptable

E. Toxicity to Plants

1. Terrestrial/Semi-aquatic

Currently, semi-aquatic plant testing is required for antifoulant pesticides on one species, rice (*Oryza sativa*).

Table 34. Tier I Toxicity of tralopryil (parent) to Rice (*Oryza sativa*)				
Test type	% a.i.	EC25, NOAEC/LOAEC, endpoints	MRID Author/ Year	Study classification
Seedling emergence	94.6	EC25 – not determined NOAEC= 0.17 mg ai/L No effects observed (percent emergence)	456741-15 Teixeira, 20041	Acceptable

2. Aquatic

Aquatic plant testing is required for antifoulant pesticides. Testing is required on one vascular plant (Lemna sp.), and four species of algae. Results of submitted studies for tralopryil and major degradates are summarized in the tables, below.

Table 35. Acute Toxicity of tralopryil (parent) to Alga and Aquatic Plants					
Species	% ai.	96-hour LC50/EC50 (ppb) (95% c.i.)	NOAEC (ppb)	MRID No. Author/ Year	Study Classification
Freshwater green alga *(Pseudokirchneriella subcapitata)*	94.6	Static EC50= 11 (10.5 – 11)	6.8	465960-06 Hoberg, 2005	Acceptable

Table 35. Acute Toxicity of tralopryil (parent) to Alga and Aquatic Plants					
Species	% ai.	96-hour LC50/EC50 (ppb) (95% c.i.)	NOAEC (ppb)	MRID No. Author/ Year	Study Classification
Freshwater green alga *(Raphidocelis subcapitata)*	94.6	Static EC50= 4.49 (1.93 – 5.05)	3.1	456741-17 Van der Kerken, 2002	Supplemental
Blue-green alga (*Anabaena flos-aquae*)	94.6	Static EC50= 350 (40 – 550)	9.2 (cell density)	458939-02 Hoberg, 2003	Acceptable
Freshwater diatom *(Navicula pelliculosa)*	94.6	Static EC50= 5.5 (5.0 – 6.2)	0.9 (cell density)	458939-03 Hoberg, 2003	Acceptable
Marine diatom *(Skeletonema costatum)*	94.6	Static EC50= 2.7 (2.6 – 2.9)	1.5	466199-01 Hoberg, 2005	Acceptable
Marine diatom *(Skeletonema costatum)*	94.6	Static EC50= 2.88	0.54	456741-18 Van der Kerken, 2002	Supplemental
Aquatic vascular plant, duckweed (*Lemna gibba*)	94.6	Static 7 day EC50 = 87.2 (59.7 – 102.6)	22	456741-16 Hoberg, 2001	Acceptable

Table 36. Acute Toxicity of CL 322,250 to Alga and Aquatic Plants

Species	% ai.	96-hour LC50/EC50 (ppb) (95% c.i.)	NOAEC (ppb)	MRID No. Author/Year	Study Classification
Freshwater green alga *(Raphidocelis subcapitata)*	93	Algal growth 96-hour EC50 >4620	1150	456741-23 Van der Kerken, 2002	Supplemental
Marine Diatom (*Skeletonema costatum*)	93	Algal growth 96-hour EC 50 = 1140 (1090 – 1190)	< 180 (EC05 = 180)	456741-24 Van der Kerken, 2002	Acceptable
Marine Diatom (*Skeletonema costatum*)	93	Algal growth 96-hour EC 50 =660	500	465960-14 Hoberg, 2005	Acceptable
Aquatic vascular plant, duckweed (*Lemna gibba*)	93	Frond density 7-day EC50 > 990	530	456741-22 Hoberg, 2001	Supplemental
Blue-green alga (*Anabaena flos-aquae*)	93	Cell density 96-hour EC50 > 830	830	458939-07 Hoberg, 2003	Supplemental
Freshwater diatom *(Navicula pelliculosa)*	93	Cell density 96-hour EC50 >930 (Stimulated growth at all treatment levels)	930	458939-08 Hoberg, 2003	Supplemental

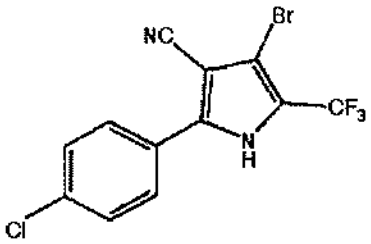
Table 37. Acute Toxicity of CL 325,195 to Alga and Aquatic Plants					
Species	**% ai.**	**96-hour LC50/EC50 (ppb) (95% c.i.)**	**NOAEC (ppb)**	**MRID No. Author/Year**	**Study Classification**
Freshwater green alga *(Raphidocelis subcapitata)*	98	Cell Density 96-hour EC50 >1990	1990	456741-26 Van der Kerken, 2002	Supplemental
Marine Diatom (*Skeletonema costatum*)	98	Algal growth 96-hour EC50 = 1200 (1120 – 1280)	160	456741-27 Van der Kerken, 2002	Supplemental
Aquatic vascular plant, duckweed (*Lemna gibba*)	98	Frond density 7-day EC50 >930	930	456741-25 Hoberg, 2001	Supplemental
Blue-green alga (*Anabaena flos-aquae*)	95.1	Cell Density 96-hour EC50 >1000	1000	458939-04 Hoberg, 2003	Supplemental
Freshwater diatom *(Navicula pelliculosa)*	95.1	Cell Density 96-hour EC50 > 980	980	458939-05 Hoberg, 2003	Supplemental

III. ENVIRONMENTAL FATE ASSESSMENT SUMMARY

(Excerpted from, "Environmental Fate Assessment for Econea™ Technical for New Chemical Registration," S.Gowda and J. Briethaupt, August 17, 2006, DP Barcode 330789) (Appendix A).

Econea™ Technical is an anti-fouling preservative that contains 93.2% of the active ingredient 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as tralopryil, AC303268 (common name), R107894, or AF028. It is used for formulation into anti-fouling products for control of hard fouling organisms such as barnacles, mussels, and polychaetes found on the hulls of boats and vessels, as well as on marine structures.

AC303268 is an off-white powder that is practically insoluble in water. The chemical structure of AC303268 is as follows:



A 45-day aqueous availability study shows that AC303268 may be released from paint into surface waters. The average leach rate of AC303268 in seawater (from Sigma Nexxium 20 Paint), between day 28 and day 45, was 8.00 $\mu g/cm^2$/day, with an average cumulative release of 12.9 $\mu g/cm^2$ through day 1 and 454 $\mu g/cm^2$ through day 45. Any AC303268 released into water is rapidly hydrolyzed, primarily at higher temperatures and pH values to one major degradate, CL 322,250 (parent minus fluorines and remaining carbon hydrated). Hydrolytically, at pH 5 and 10°C, the half-life of AC303268 is 168 days, as opposed to 15 days at pH 5 and 25°C, and less than 3 days at pH 7 and pH 9 (10 and 25°C). In seawater, AC303268 hydrolyzes with a half-life of less than 1 day at 10 and 25°C. The degradate CL 322,250 does not degrade at any pH or temperature due to hydrolysis. Based on its rapid hydrolysis, AC303268 may not pose a concern as a contaminant in surface waters. However, because of its stability, CL 322,250 may be a concern.

Aerobic and anaerobic aquatic metabolism continue to degrade AC303268, decreasing the threat of surface water contamination. In an aerobic aquatic metabolism study, AC303268 degraded with estimated half-lives of 3-7 days and less than 1 day in freshwater and marine test systems, respectively. Two major degradates, CL 322,250 and debrominated CL 322,250 (found only in marine water), were identified and the majority of the residues were found in the aqueous layer, as opposed to the sediment. CL 322,250 was stable in the freshwater test system and degraded with a half-life of 288 days in the marine test system. Under anaerobic conditions, AC303268 degraded into the same two degradates in both the freshwater and marine test systems, and were again found primarily in the aqueous layer. Half-lives were similar at 10 days in the freshwater test system and 0.03 days in the marine test system. However, the percent of degradate present during different periods of time varies with the type of metabolism. In addition, CL 322,250 continued to degrade (half-lives 31 and 22 days) to debrominated 322,250 in the freshwater and marine test system under anaerobic conditions.

AC303268 is also expected to absorb to suspended solids and sediments in surface waters, thereby reducing its concentration in surface waters. In a batch equilibrium study, an average of 98.89 and 98.38% of the applied amount was absorbed in the freshwater soils (sandy loam and silt loam), respectively. In marine soils (sand and loam), an average of 83.18% and 97.48% was absorbed, respectively. Average adsorption K_d values ranged from 450 to 335 ml/g in the freshwater soils and from 26 to 196 ml/g in the marine soils. Corresponding K_{oc} values were 20440 to 16733 and 3582 to 5588 ml/g. Desorption K_d and K_{oc} values were higher than those obtained for adsorption. Adorption coefficients for the degradate CL 322,250 indicate that

it is also absorbed to suspended solids and sediments.

The estimated Log Kow for parent Econea™ (AC 303268) is 3.0, and the estimated Log Kow values for the primary degradate (CL 322,250) are 1.66 in freshwater and 0.55 in salt water. Parent Econea™ generally degrades quickly in water to CL 322,250, and therefore bioconcentration was modeled using the primary degradate. A Log Kow of less than 3.0 (Kow <1000) would be indicative of bioconcentration that is below our level of concern. Therefore, significant bioconcentration of CL 322,250 in freshwater and saltwater fish is not likely to occur. The Agency has estimated bioconcentration factors (BCFs) of 11X (pH 6) and 3X (pH8) in freshwater and seawater, respectively.

IV. ENVIRONMENTAL EXPOSURE ASSESSMENT SUMMARY (Excerpted from, "Estimated Environmental Concentrations for ECONEA™ Antifoulant Agent," S. Mostaghimi, November 15, 2006, DP Barcode 330451) (Appendix B).

The following is a summary of the results from modeling data which were submitted by the Janssen Pharmacetuica Inc. in a submission titled "Environmental and Ecological Risk Assessment of ECONEA Antifoulant Agent (MRID# 468466-03)" . The inputs used for running MAM-PEC (Marine Antifoulant Model to predict Environmental Concentrations) and, EFDC (Environmental Fluid Dynamic Code), appear correct and the data reported from the runs are acceptable. The inputs for the TRIM2D (Tidal Residual Inter-tidal Mudflat) appear correct; however, the outputs from this model run could not be verified independently because of the licensing issues and the lack of availability of TRIM2D algorithms to the public.

MAM-PEC is used as an assessment tool for antifoulant risk assessments in Europe. MAM-PEC was developed by the Institute of Environmental Studies/IVM and Delft Hydraulics for the European Paint Makers Association (CEPE) for conducting risk assessments for antifouling agents. The model provides prediction of environmental concentrations of antifouling products in six generalized "typical" marine environments (commercial harbor, estuarine harbor, marina, marina poorly flushed, open sea, and shipping lane).

FEDC is a multifunctional surface water modeling system, which includes hydrodynamic, sediment-contaminant, and eutrophication components. The EFDC model is capable of 1, 2, and 3-D spatial resolution. The model uses a curvilinear-orthogonal horizontal grid and a sigma terrain following vertical grid. The EFDC model can represent the transport and fate of an arbitrary number of contaminants, including metals and hydrophobic organics, sorbed to any of the sediment classes and dissolved and particulate organic carbon using a three-phase equilibrium partitioning formulation. The public domain EFDC program was originally developed at the Virginia Institute of Marine Science and is currently maintained by Tetra Tech, Inc. with support from the US EPA.

R107894 breaks down rapidly in the environment. Degradation from aqueous hydrolysis has been reported to occur with half-lives of 3 and 15 hours in seawater (at temperatures of 25° and 10° C, respectively), and 2 and 12 hours in freshwater at pH 7 (25° and 10°C, respectively). Half-lives of 2 to 4 days in water have been reported in marine and freshwater aerobic aquatic metabolism studies. Half-lives in sediment or full test system were longer in those studies (31 and 13 days, respectively).

Degradation products include CL322250 and CL325195. CL322250 breaks down further to form CL322248. Maximum formation (percent of R107894) observed in marine aerobic aquatic metabolism studies have been 70, 76, and 7 percent for CL322250, CL322248, and CL325195, respectively.

The study submitted by the registrant focuses on CL322250 and CL322248 based on their expected respective rates of formation, persistence, toxicity, and potential for toxicological effects in the environment. **R107894 is not addressed because of its rapid degradation in the environment and low potential for bioaccumulation. CL322195 is not addressed based on its relatively low rate of formation and low toxicity to test species.**

Model simulations were used to estimate the concentrations of the CL322250 and CL322248 in five harbor system in the United States. The systems modeled, models used and the rationale for use of the models are presented in the table, below.

Table 38. Models used for estimating environmental concentrations of ECONEA ™ in different systems.

System	Model	Rationale
Commercial, Estuarine, Marina, Marina Poorly Flushed, Shipping Lane, and Open Sea	MAM-PEC	Screening level assessment using standard environments developed for the European Union.
Barbours Cut – Houston	MAM-PEC	Screening level representation of harbor system developed for this study.
Baltimore Harbor	MAM-PEC	Screening level representation of harbor system developed for this study.
Norfolk Harbor/James River	EFDC	Detailed representation of harbor system previously setup by VIMS.
Port of New Orleans, lower Mississippi River	EFDC	Detailed representation of harbor system developed for this study.

San Diego Bay	TRIM2D	Detailed representation of harbor system previously developed by SSC SD.

The estimated environmental concentrations for CL322250 and CL322248 from MAM-PEC runs in Baltimore and Barbarous Point Houston are shown in the table, below. Both maximum and average concentration in water column and sediments are presented in this table.

Table 39. Maximum and Average concentrations of CL322250 and CL322248 in Baltimore harbor and Barbours Point Houston estimated by MAM-PEC model.

Chemical	Statistics	Location			
		Baltimore		Barbours Point Houston	
		Water µg/l	Sediment (µg/g dw)	Water µg/l	Sediment (µg/g dw)
CL322250	Maximum	0.041	7.77E-5	0.448	8.44E-4
	Average	0.024	4.51E-5	0.335	6.32E-4
CL322248	Maximum	0.037	1.54E-4	0.406	1.66E-3
	Average	0.022	8.92E-5	0.304	1.24E-3

The data from MAP-PEC result for the Barbours Point Houston in water should be used for the ecological risk assessment. It should be noted that the highest concentrations were reported in San Diego Harbor by the TRIMD2 model. However, because of the lack of enough information for the TRIMD2 model the data from this model could not be verified independently.

V. Environmental Risk Assessment and Risk Characterization

Exposure and Risk to Nontarget Terrestrial Animals and Aquatic Organisms

Risk characterization integrates the results of the exposure and ecotoxicity data to evaluate the likelihood of adverse ecological effects. The means of this integration is called the quotient method. Risk quotients (RQs) are calculated by dividing exposure estimates by acute and chronic ecotoxicity values. RQ = EXPOSURE/TOXICITY

RQs are then compared to OPP's levels of concern (LOCs). These LOCs are used by OPP to analyze potential risk to nontarget organisms and the need to consider regulatory action. The criteria indicate that a pesticide used as directed has the potential to cause adverse effects on nontarget organisms. LOCs currently address the following risk presumption categories: (1) **acute** -- potential for acute risk to non-target organisms which may warrant regulatory action in addition to restricted use classification, (2) **acute restricted use** -- the potential for acute risk to non-target organisms, but may be mitigated through restricted use classification, (3) **acute endangered species** - endangered species may be adversely affected by use, (4) **chronic risk** - the potential for chronic risk may warrant regulatory action, endangered species may potentially be affected through chronic exposure, (5) **non-endangered plant risk** - potential for effects in

non-target plants, and (6) **endangered plant risk** – potential for effects in endangered plants. Currently, AD does not perform assessments for chronic risk to plants, acute or chronic risks to nontarget insects, or chronic risk from granular/bait formulations to birds or mammals.

The ecotoxicity test values (measurement endpoints) used in the acute and chronic risk quotients are derived from required studies. Examples of ecotoxicity values derived from short-term laboratory studies that assess acute effects are: (1) LC_{50} (fish and birds), (2) LD_{50} (birds and mammals), (3) EC_{50} (aquatic plants and aquatic invertebrates) and (4) EC_{25} (terrestrial plants). Examples of toxicity test effect levels derived from the results of long-term laboratory studies that assess chronic effects are: (1) LOAEC (birds, fish, and aquatic invertebrates), and (2) NOAEC (birds, fish and aquatic invertebrates). For birds and mammals, the NOAEC generally is used as the ecotoxicity test value in assessing chronic effects, although other values may be used when justified. However, the NOAEC is used if the measurement endpoint is production of offspring or survival.

Risk presumptions and the corresponding RQs and LOCs are tabulated below.

Table 1. Risk Presumption Categories

Risk Presumption for Terrestrial Animals	**LOC**
Acute: Potential for acute risk for all non-target organisms	>0.5
Acute Restricted Use: Potential for acute risk for all non-target organisms, but may be mitigated through restricted use classification	>0.2
Acute Endangered Species: endangered species may be adversely affected by use	>0.1
Chronic Risk: potential for chronic risk may warrant regulatory action	>1
Risk Presumption for Aquatic Organisms	**LOC**
Acute: Potential for acute risk for all non-target organisms	>0.5
Acute Restricted Use: Potential for acute risk for all non-target organisms, but may be mitigated through restricted use classification	>0.1
Acute Endangered Species: endangered species may be adversely affected by use	>0.05
Chronic Risk: potential for chronic risk may warrant regulatory action	>1
Risk Presumption for Terrestrial and Aquatic Plants	**LOC**
Potential for risk for all non-endangered and endangered plants	>1

A. Environmental Risk Assessment for Terrestrial Organisms:

Terrestrial exposure is not modeled for aquatic uses such as antifoulants. However, there is concern for waterfowl and other aquatic birds and mammals for exposure to tralopyril or its

degradates because tralopryil is the primary degradate of chlorfenapry (Pirate) insecticide. Chlorfenapyr is currently not registered for use on agricultural crops, but is restricted to indoor uses only based on very high toxicity to birds and aquatic organisms. Studies in Agency files for chlorfenapry were reviewed. Parent tralopryil breaks down rapidly in water to form CL322,250, in certain cases CL322,248, and to a very limited extent CL325,195. Concern for exposure to birds is primarily from CL322,250 and CL322,248. EECs from the Barbours Point Marina (maximum expected environmental concentrations described in the environmental exposure section, above) for CL322,250 and CL322,248 were compared to avian dietary toxicity endpoints as a screening-level estimate of risk:

Table 40: Risk Quotients for Waterfowl Exposed to CL322,250 and CL322,248

Compound	Avian Dietary LC50	EEC	RQ
CL322,250	250 ppm	Max: 0.448 ppb Avg: 0.335 ppb	Max: 0 Avg: 0
CL322,248	3160 ppm	Max: 0.406 ppb Avg: 0.304 ppb	Max: 0 Avg: 0

Based on the above risk quotients, adverse effects from exposure to the major degradates of Econea™ are not likely expected. No Levels of Concern (LOCs) were exceeded for the maximum exposure marina scenario. Using the EECs in this manner assumes that the birds would be exposed through ingestion of the contaminated water. The studies from which the toxicity endpoints were derived exposed the birds through feed, not drinking water. It is unknown if the same mechanisms of toxicity would occur via drinking water exposure. No validated model was available to determine the exposure to birds via food items from the use of an antifouling compound.

B. Environmental Risk Assessment for Aquatic Organisms:

As discussed in the environmental fate and exposure sections, above, the parent compound in Econea™ (tralopryil), while highly toxic to aquatic organisms, rapidly degrades (within several hours) to CL322,250 in salt and freshwater. Under certain conditions, CL322,250 can further degrade to CL322,248 and to a minor extent to CL325,195. The exposure and risk assessments focus on the two primary degradates CL 322,250 and CL322,248, rather than the parent compound. To develop RQs, the EECs from Barbours Point Marina were compared to the most-sensitive endpoint for each taxa. Maximum and average values were used to develop maximum and average RQs. The Barbours Point marina scenario was modeled using MAM-PEC (Marine Antifouling Model to Predict Environmental Concentrations). MAM-PEC is used as an assessment tool for antifoulant risk assessments in Europe and is undergoing OECD review for worldwide acceptance. MAM-PEC was developed by the Institute of Environmental Studies/IVM and Delft Hydraulics for the European Paint Makers Association (CEPE) for conducting risk assessments for antifouling agents. The model provides predictions of

environmental concentrations of antifouling products in six generalized "typical" marine environments (commercial harbor, estuarine harbor, marina, marina poorly flushed, open sea, and shipping lane).

Barbour's Point Marina: The Port of Houston is the sixth largest seaport in the world, the second busiest port in the United States, and the eighth in container ship traffic (U.S. Dept. of Transportation, 2004). Barbours Cut is the premier container terminal in the Port of Houston and handles the majority of the container traffic. Each year there are over 6,400 vessel calls to the port handling more foreign waterborne tonnage than any other U.S. port. The port is distributed along the 50-mile Houston Ship Channel and contains numerous terminals and wharves for the large and diverse number of ship types that call on the port. The Barbours Cut Terminal in the Port of Houston (Figure 4) was chosen as a realistic worst-case scenario to evaluate emissions of biocides for the following reasons:

- The Port of Houston handles 64% of the containerized cargo market in the U.S. Barbours Cut is the port's premier container terminal and handles much of this traffic.
- Barbours Cut Terminal has the most modernized and efficient cargo handling system in the Gulf of Mexico and was designed for fast ship turn around and has the ability to handle a large amount of ship traffic.
- Barbours Cut is relatively shallow (12.8 m) and is relatively small for the amount of traffic that it sees.
- Since Barbours Cut is a container ship terminal, it serves relatively large ships with large anti-fouled areas.
- Barbours Cut has a long narrow mouth entering into the harbor, limiting the amount of tidal flushing between the harbor and the shipping channel.

The MAM-PEC model was used to simulate Baltimore Harbor and Barbours Cut, the EFDC model (Tetra Tech and Virginia Institute of Marine Sciences) was used to simulate Norfolk Harbor/James River, and the Navy's TRIM2D model was used to simulate residues in San Diego Bay. The MAM-PEC modeling runs of Barbours Cut resulted in the highest residue levels of all areas modeled, thus they were used to assess risk in the risk quotients below. Predicted levels of CL322,250 and CL322,248 in water and sediment were used in the following tables:

Table 41: Aquatic Organism Risk Quotients for Antifoulant Uses of Econea™

Taxa/Endpoint	Barbours Point CL322,250 max	Barbours Point CL322,250 avg	Barbours Point CL322,248 max	Barbours Point CL322,248 avg
Water Column EECs	***0.448 ppb***	***0.335 ppb***	***0.406 ppb***	***0.304 ppb***
Freshwater fish Acute CL322,250 N/A CL322,248 N/A				
Freshwater Invertebrates Acute CL322,250 EC50 = 700 ppb CL322,248 EC50 = 16800 ppb	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Marine/Estuarine Fish Acute CL322,250 LC50 >950 ppb CL322,248 LC50 >89000	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Marine/Estuarine Bivalve Acute CL322,250 EC50 = 310 ppb CL322,248 n/a	0.00	0.00	0.00	0.00
Marine/Estuarine Invertebrate Acute CL322,250 LC50 = 550 ppb CL322,248 LC50 = 4300 ppb	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Rice NOEC (parent) 170 ppb	0.00	0.00	0.00	0.00
FW green Algae CL322,250 EC50 >4620 ppb NOEC=1150 ppb	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00

Blue-green Alga CL322,250 EC50 >830 ppb NOEC=830 ppb	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Marine diatom CL322,250 EC50 >1140 ppb NOEC <180 ppb	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Freshwater diatom CL322,250 EC50 >930 ppb NOEC=930 ppb	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Aquatic vascular plant CL322,250 EC50 >990 ppb NOEC=530 ppb	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Plant testing N/A for CL322,248				
Fish Chronic - Freshwater CL322,250 NOEC = 69 ppb CL322,248 N/A	0.01	0.00	0.00	0.00
Fish Chronic - Marine CL322,250 NOEC = 240 ppb CL322,248 N/A	0.00	0.00	0.00	0.00
Invertebrate Chronic - Freshwater CL322,250 NOEC = 300 ppb CL322,248 NOEC = 1370	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Invertebrate Chronic - Marine CL322,250 N/A CL322,248 N/A				
Sediment EECS	***0.000844 µg/g***	***0.000632 µg/g***	***0.00166 µg/g***	***0.00124 µg/g***

Sediment toxicity – freshwater CL322,250 LC50>35 mg/kg CL322,248 N/A	0.00	0.00	0.00	0.00
Sediment toxicity - marine CL322,250 LC50 > 70 mg/kg CL322,248 LC50 >75 mg/kg	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00

Acute RQs for freshwater fish could not be calculated as no valid freshwater fish acute toxicity data for either major degradate were available. If data on the parent compound were used, the resulting RQs would exceed high risk LOCs for the maximum concentration, and restricted use LOCs for the average concentration. However, parent tralopryil rapidly breaks down in fresh water to CL322,250, which has been demonstrated to be substantially less toxic to marine fish and freshwater and marine invertebrates than the parent compound in submitted studies. Submitted freshwater fish acute toxicity studies for the degradates, which were not acceptable to the Agency, did demonstrate that the LC50s for each degradate is significantly less toxic than the parent compound (520 ppb and >2710 ppb for CL322,250 and CL322,248, respectively). **Re-testing with CL322,250 on the more sensitive species (rainbow trout, *Oncorhynchus mykiss*) is required as confirmatory data.**

Based on the environmental modeling results for Barbours Point marina, risks to aquatic organisms from maximum water column and sediment concentrations are well below the Agency's LOCs, including the Endangered Species LOC. Kows for tralopryil, as well as the major degradate CL322,250, are low, and estimated BCFs for CL322,250 are also low, indicating low potential for bioaccumulation in aquatic organisms.

C. Endangered Species Considerations

Section 7 of the Endangered Species Act, 16 U.S.C. Section 1536(a)(2), requires all federal agencies to consult with the National Marine Fisheries Service (NMFS) for marine and anadromous listed species, or the United States Fish and Wildlife Services (FWS) for listed wildlife and freshwater organisms, if they are proposing an "action" that may affect listed species or their designated habitat. Each federal agency is required under the Act to insure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat. To jeopardize the continued existence of a listed species means "to engage in an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of the species." 50 C.F.R. ' 402.02.

To facilitate compliance with the requirements of the Endangered Species Act subsection (a)(2) the Environmental Protection Agency, Office of Pesticide Programs has established procedures to evaluate whether a proposed registration action may directly or indirectly reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of any listed species (U.S. EPA 2004). After the Agency's screening-level risk assessment is performed, if any of the Agency's Listed Species LOC Criteria are exceeded for either direct or indirect effects, a determination is made to identify if any listed or candidate species may co-occur in the area of the proposed pesticide use. If determined that listed or candidate species may be present in the proposed use areas, further biological assessment is undertaken. The extent to which listed species may be at risk then determines the need for the development of a more comprehensive consultation package as required by the Endangered Species Act.

A literature search was not conducted, however, an ECOTOX search is ongoing to determine if additional toxicity or exposure endpoints are available.

Based on the environmental modeling for the Barbours Point marina, which represents the highest risk potential of the scenarios modeled, there is minimal non-target risk to aquatic organisms or waterfowl from the proposed use of Econea™ as an antifoulant. Exposure to non-target terrestrial organisms is also expected to be minimal. The only area having potential for very limited acute toxicity to aquatic organisms is a fresh/clear water dockage having little or no water exchange around a ship that is docked for a long period of time. Econea areas of exposure may overlap with listed species which warrants a more refined assessment, to include indirect and habitat effects. A more refined assessment involves clear delineation of the action area associated with proposed use of Econea antifoulant and use of best available information on the temporal and spatial co-location of listed species with respect to the action area. Because a refined risk assessment has not been conducted for this action an endangered species effect determination will not be made at this time.

Outstanding data for degradate CL322,250:

850.1075/72-1 Freshwater fish acute toxicity testing with coldwater species (Rainbow trout)

Labeling Hazard Statement:

"Tralopryil is toxic to birds, fish, aquatic invertebrates, shrimp, oysters and clams".

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Appendix A: Environmental Fate Science Chapter for Econea™

ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION,
PESTICIDES AND
TOXIC

SUBSTANCES

August 17, 2006

MEMORANDUM

SUBJECT: Environmental Fate Assessment of Econea™ Technical for New Chemical Registration

Case No.: **DP Barcode:** 330789

FROM: Srinivas Gowda, Microbiologist/Chemist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

James Breithaupt, Agronomist
Environmental Risk Branch II
Environmental Fate and Effects Division (7507P)

TO: Marshall Swindell, Team Leader
Karen Leavy, Risk Manager Reviewer
Regulatory Management Branch I
Antimicrobials Division (7510P)

THRU: Siroos Mostaghimi, Team Leader, Team one
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Norman Cook, Branch Chief
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Chemical Name	PC Code	CAS#	Common Name

1H-Pyrrole-3-Carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-	119093	122454-29-9	Econea™

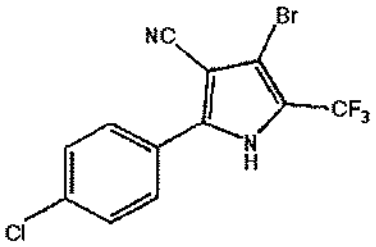
Environmental Fate Science Chapter and Fate Assessment on Econea™ Technical is submitted for New Chemical Registration.

ECONEA™ Technical
ENVIRONMENTAL FATE SCIENCE CHAPTER

EXECUTIVE SUMMARY

ECONEA™ Technical is an anti-fouling preservative that contains 93.2% of the active ingredient 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as AC303268 (common name), R107894, or AF028. It is used for formulation into anti-fouling products for control of hard fouling organisms such as barnacles, mussels, and polychaetes found on the hulls of boats and vessels, as well as on marine structures.

AC303268 is an off-white powder that is practically insoluble in water. The chemical structure of AC303268 is as follows:



A 45-day aqueous availability study shows that AC303268 may be released from paint into surface waters. The average leach rate of AC303268 in seawater (from Sigma Nexxium 20 Paint), between day 28 and day 45, was 8.00 $\mu g/cm^2$/day, with an average cumulative release of 12.9 $\mu g/cm^2$ through day 1 and 454 $\mu g/cm^2$ through day 45. Any AC303268 released into water is rapidly hydrolyzed, primarily at higher temperatures and pH values to one major degradate, CL 322,250 (parent minus fluorines and remaining carbon hydrated). Hydrolytically, at pH 5 and 10°C, the half-life of AC303268 is 168 days, as opposed to 15 days at pH 5 and 25°C, and less than 3 days at pH 7 and pH 9 (10 and 25°C). In seawater, AC303268 hydrolyzes with a half-life of less than 1 day at 10 and 25°C. The degradate CL 322,250 does not degrade at any pH or temperature due to hydrolysis. Based on its rapid hydrolysis, AC303268 may not pose a concern as a contaminant in surface waters. However, because of its stability, CL 322,250 may be a concern.

Aerobic and anaerobic aquatic metabolism continue to degrade AC303268, decreasing the threat of surface water contamination. In an aerobic aquatic metabolism study, AC303268

degraded with estimated half-lives of 3-7 days and less than 1 day in freshwater and marine test systems, respectively. Two major degradates, CL 322,250 and debrominated CL 322,250 (found only in marine water), were identified and the majority of the residues were found in the aqueous layer, as opposed to the sediment. CL 322,250 was stable in the freshwater test system and degraded with a half-life of 288 days in the marine test system. Under anaerobic conditions, AC303268 degraded into the same two degradates in both the freshwater and marine test systems, and were again found primarily in the aqueous layer. Half-lives were similar at 10 days in the freshwater test system and 0.03 days in the marine test system. However, the percent of degradate present during different periods of time varies with the type of metabolism. In addition, CL 322,250 continued to degrade (half-lives 31 and 22 days) to debrominated 322,250 in the freshwater and marine test system under anaerobic conditions.

AC303268 is also expected to absorb to suspended solids and sediments in surface waters, thereby reducing its concentration in surface waters. In a batch equilibrium study, an average of 98.89 and 98.38% of the applied amount was absorbed in the freshwater soils (sandy loam and silt loam), respectively. In marine soils (sand and loam), an average of 83.18% and 97.48% was absorbed, respectively. Average adsorption K_d values ranged from 450 to 335 ml/g in the freshwater soils and from 26 to 196 ml/g in the marine soils. Corresponding K_{oc} values were 20440 to 16733 and 3582 to 5588 ml/g. Desorption K_d and K_{oc} values were higher than those obtained for adsorption. Adorption coefficients for the degradate CL 322,250 indicate that it is also absorbed to suspended solids and sediments.

The estimated Log Kow for parent Econea™ (AC 303268) is 3.0, and the estimated Log Kow values for the primary degradate (CL 322,250) are 1.66 in freshwater and 0.55 in salt water. Parent Econea™ generally degrades quickly in water to CL 322,250, and therefore bioconcentration was modeled using the primary degradate. A Log Kow of less than 3.0 (Kow <1000) would be indicative of bioconcentration that is below our level of concern. Therefore, significant bioconcentration of CL 322,250 in freshwater and saltwater fish is not likely to occur. The Agency has estimated bioconcentration factors (BCFs) of 11X (pH 6) and 3X (pH8) in freshwater and seawater, respectively.

I. Environmental Fate Assessment

A. Abiotic

In a hydrolysis study conducted under abiotic and buffered conditions, AC303268 (R107894) was rapidly hydrolyzed, primarily at higher temperatures and pH values. The study was conducted in the dark at temperatures of 10 and 25 ± 1°C for up to 30 days at pH 5, pH 7, pH 9, and in synthetic seawater (pH 8-nonbuffered). At 25°C, AC303268 hydrolyzed with respective half-lives of 15 days, 8 hours, 2 hours and 3 hours at pH 5, pH 7, pH 9 and in seawater. Half-lives were 168 days, 69 hours, 12 hours and 15 hours at 10°C. Hydrolysis produced CL 322,250 as the major degradate, which was present in all solutions analyzed with the exception of the pH 5 solution at 10°C. Traces of CL 325,195 (hydrated and debrominated

parent) were also identified. Only minor hydrolytic products were formed in the pH 5 solution at 10°C. At 10°C, CL 322,250 was present at a maximum concentration of 72.7% of the applied (day 21) and at a maximum concentration of 96.2% (day 30) in the pH 7 and pH 9 buffered solutions, respectively. In seawater, a maximum concentration of 95.8% of the applied was observed on day 21. At 25°C, CL 322,250 was present at maximum concentrations of 73.9% (day 30), 72.4% (day 7), 96.9% (day 7), and 96.3% (24 hrs) of the applied in the pH 5, pH 7, pH 9 and seawater test solutions, respectively. The hydrolysis guideline requirements (OPPTS 161-1) for ECONEA™ Technical have been fulfilled by this study (MRID Nos. 456739-08 and 456739-09).

The Agency also performed regression analyses using the data presented in the study to estimate the half-lives of the parent compound (AC303268) and the major degradate (CL 322,250). In freshwater, half-lives of the parent compound ranged from 177 days at pH 5 and 10°C, 15 days at pH 5 and 25°C, to 3 days in the pH 7 and 9 buffered solutions at 10 and 25°C. The half-lives were less than 1 day in the seawater (pH 8) at 10 and 25°C. While degradation of the parent compound occurred, CL 322,250 did not degrade at any pH or temperature.

A 45-day aqueous availability study determined the rate at which two active ingredients, one of which was AC303268 (AF028), are released from Sigma Nexxium 20 Paint. The paint was applied to polycarbonate cylinders which were immersed in a tank with continuously pumped synthetic seawater. The average leach rate between day 28 and day 45 was 8.00 $\mu g/cm^2$/day. The average cumulative release was 12.9 $\mu g/cm^2$ through day 1 and 454 $\mu g/cm^2$ through day 45. The study reflects the guideline specified for the ASTM Standard Test Method D5108-90 for aqueous availability (MRID No. 456732-01).

B. Biotic

The aerobic metabolism of AC303268 (R107894) was studied in a natural freshwater/sediment system (water pH 6.5, silt loam, organic carbon 2.5%) and a natural marine water/sediment system (water pH 8.04, sandy loam, organic carbon 0.8%). The study was conducted for 30 days in the dark at 21°C. AC303268 was applied at the rate of 0.5 mg/L. The estimated half-life (based on visual inspection of the data) in the freshwater system was between 3 and 7 days. In the marine system, the half-life was estimated as being less than 1 day. The two major degradates identified were CL 322,250 and debrominated CL 322,250. There were also four minor degradates. A higher percentage of both the parent compound and the degradates was found in the aqueous phase as opposed to the sediment. The major degradate identified in the freshwater was CL 322,250, with a maximum concentration of 48.2% of the applied on day 7. The major degradate in the freshwater sediment was also CL 322,250, with a maximum concentration of 7.85% of the applied observed on the last day (30^{th}) of the study. There were two major degradates identified in the marine water and sediment. CL 322,250 and debrominated CL 322,250 were detected in the marine water at maximum concentrations of 71.9% and 19.5% of the applied, respectively, on days 7 and 30 of the study. In the marine sediment, CL 322,250

and debrominated CL 322,250 were detected at maximum concentrations of 5.22% and 10.8% of the applied on days 15 and 30, respectively. The aerobic aquatic metabolism guideline requirements (OPPTS 162-4) for ECONEA™ Technical have been fulfilled by this study (MRID Nos. 456739-11 and 456739-12.

The Agency also performed regression analyses using the data presented in the study to estimate the half-lives of the parent compound (AC303268) and the major degradate (CL 322,250). In the freshwater system, the half-life of the parent compound was estimated at 12 days. CL 322,250 was stable. The half-lives were 0.62 and 288 days, respectively, for the parent compound and CL 322,250 in the marine system, where CL 322,250 farther degraded to debrominated CL 322,250.

A study of the anaerobic metabolism of AC303268 (R107894) was also performed. The study was conducted in a natural freshwater/sediment system (water pH 5.8, silt loam, organic carbon 2.5%) and a marine water/sediment system (water pH 7.7, loamy sand), organic carbon 0.8%) for 52 weeks in the dark at 21°C. AC303268 was applied at the rate of 69 µg/L. Based on modeling, AC303268 degraded with a half-life of 10 days in the freshwater system and a half-life of 0.03 days in the marine system. The major degradates of both the freshwater system and the marine system were CL 322,250 and CL 325,195 (hydrated and debrominated parent). Seven unknown minor degradates were also detected. In the water of freshwater test system, CL 322,250 was present at a maximum concentration of 44.10% of the applied on day 14. CL 325,195 was below the detection limit throughout the study period. In the water of the marine test system, CL 322,250 and CL 325,195 were at maximum concentrations of 60.34% and 6.64% of the applied, respectively, on day 3. Maximum concentrations in the sediment of the freshwater system were 10.05% of the applied for CL 322,250 and 1.29% of the applied for CL 325,195, observed on day 14 and day 7, respectively. In the marine system, maximum concentrations in the sediment were 16.35% of the applied on day 7 and 1.39% of the applied at time 0. This study satisfies the anaerobic metabolism guideline requirements for ECONEA™ Technical (OPPTS 162-3) (MRID No. 456739-10).

The Agency also performed regression analyses using the data presented in the study to estimate the half-lives of the parent compound (AC303268) and the major degradate (CL 322,250). In the freshwater system, half-lives of the parent and the major degradate were 29 and 31 days, respectively. The half-lives were 0.68 and 22 days, respectively, for the parent compound and CL 322,250 in the marine system.

The adsorption/desorption characteristics of AC303268 (R107894) were studied in two freshwater soils, sandy loam and silt loam, and two marine soils, sand and loam. Results of the study indicate that AC303268 is strongly absorbed to soil. After 4 hrs of equilibration for sandy loam, silt loam, loam and 8 hrs of equilibration for sand, an average of 98.89, 98.38, 97.48 and 83.18% of the applied amount was adsorbed, respectively. Average adsorption K_d values were 450, 335, 26, and 196 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. The average adsorption K_{oc} values were 20440, 16733, 3582, and 5588 ml/g in sandy loam, silt loam,

sand, and loam soils, respectively. K_f values were 446, 349, 22, and 183 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. At the end of the desorption phase, 0.84, 0.88, 9.62, and 1.63% of the adsorbed AC303268 was desorbed in the sandy loam, silt loam, sand, and loam soils, respectively. Average desorption K_d values were 599, 568, 40, and 299 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. The average desorption K_{oc} values were 27229, 28353, 5658, and 8543 ml/g for sandy loam, silt loam, sand, and loam soils, respectively. Desorption K_d and K_{oc} values were higher than those obtained for adsorption. The adsorption/desorption guidelines requirements (OPPTS 163-1) for ECONEA™ Technical have been fulfilled by this study (MRID No. 456739-13).

The adsorption/desorption properties of the parent compound (AC 303268) and the major degradate (CL 322,250) were also estimated by the Agency using the data presented in the study. Adsorption K_f values (parent compound) of 446 and 349 ml/g were estimated for the freshwater soils (sandy loam and silt loam) and K_f values of 22 and 183 ml/g were estimated for the marine soils (sand and loam). Corresponding K_{oc} values were 20273, 17450, 3143 and 5229 ml/g. No correlation with clay, organic matter, or pH was noted. The adsorption and desorption coefficients of the degradate CL 322,250 were similar. Adsorption K_f values of 189 and 357 were estimated for the freshwater soils. The adsorption K_f values in marine soils were 14 and 119. Corresponding K_{oc} values were 8591, 17850, 2000, and 3400 ml/g. As with the parent compound, desorption K_f and K_{oc} values for CL 322,250 were higher in all soils.

The bioconcentration of the major degradate CL 322,250 in freshwater and seawater was estimated by Agency based on the log octanol/water partition coefficient (Log Kow). Using equations presented in the OECD TG 305 Guideline, bioconcentration factors of 11X (pH 6) and 3X (pH8) were predicted in freshwater and saltwater fish, respectively.

APPENDIX

Environmental Fate Data for ECONEA™ Technical

A. Environmental Fate Guideline Studies

1. Hydrolysis (Guideline Number OPPTS 161-1, MRID No. 456739-08 and 456739-09)

This hydrolysis study, submitted under MRID Nos. 456739-08 and 456739-09, was reviewed by the Agency and found to be acceptable for the active ingredient, 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as R107894. The hydrolysis data requirements for ECONEA™ Technical have been fulfilled.

In the main part of the study (MRID No. 456739-08), hydrolysis of radiolabelled [^{14}C]-R107894, at a nominal concentration of 0.5 µg/g, was studied. The test solutions were incubated in the dark at nominal temperatures of 10 and 25 ± 1EC for up to 30 days in 0.01 M citrate buffer (pH 5), 0.01 M TRIS-maleic acid buffer (pH 7), 0.01 M borate buffer (pH 9) and seawater. Samples were analyzed at 0, 3, 5, 12, and 24 hours and at 2, 3, 4, 7, 10, 14, 21, and 30 days. Radioactivity was quantified by direct injection using a liquid scintillation analyzer (Packard Tri-carb 1600 TR) and identification of the transformation products was conducted using high performance liquid chromatography (HPLC) (Hewlett-Packard 1050 series HPLC and a Berthold LB 507A radioactivity monitor) and thin layer chromatography (TLC) (Molecular Dynamics phosphor imager).

The radioactive balance was 87.2 ± 11.8%, 88.6 ± 2.0%, 102.2 ± 1.0%, and 87.1 ± 0.8% of the applied at pH 5, pH 7, pH 9, and seawater at 10EC, respectively. At test termination, the concentration of the parent compound at 10EC decreased from 94.0% at day 0 to 80.9% of the initial at pH 5, decreased from 77.9% of the initial at day 0 to not detectable by day 21 at pH 7, decreased from 51.4% of the initial at day 0 to not detectable by day 4 at pH 9, and decreased from 54.9% of the initial at day 0 to not detectable by day 4 in seawater. At pH 5 (10EC) there were no major transformation products detected. At pH 7 (10EC), the major transformation products detected were CL 322,250 and Unknown B with maximum concentrations of 72.7% and 25.8% of the applied observed on the 21st and 30th days of incubation, respectively. At pH 9, the major transformation product detected was CL 322,250, with a maximum concentration of 96.2% of the applied amount observed on the 30th day of incubation. In seawater, the major transformation product detected was CL 322,250 with a maximum concentration of 95.8% of the applied amount observed on the 21st day of incubation. The minor transformation products detected at pH 5 were CL 322,250; CL 325,195; Unknown C; Unknown D; and Unknown G formed at maximum concentrations of 9.4, 4.2, 3.1, 2.6, and 0.61% of the applied, respectively. The minor transformation products detected at pH 7 were CL 325,195; Unknown A; Unknown C; and Unknown D formed at maximum concentrations of 1.6, 5.8, 1.8, and 1.9% of the applied, respectively. The minor transformation products detected at pH 9 were CL 325,195;

Unknown A; Unknown B; Unknown C; and Unknown D formed at maximum concentrations of 2.7, 1.4, 2.0, 1.4, and 1.8% of the applied, respectively. The minor transformation products detected in seawater were CL 325,195; Unknown A; Unknown C; and Unknown D formed at maximum concentrations of 2.8, 1.3, 1.7, and 1.9% of the applied, respectively. Volatiles were not formed.

The radioactive balance was 100.7 ± 2.2%, 89.6 ± 1.4%, 102.6 ± 1.3%, and 89.0 ± 1.2% of the applied at pH 5, pH 7, pH 9, and seawater at 25EC, respectively. At test termination, the concentration of the parent compound at 25EC decreased from 93.3% at day 0 to 22.2% of the initial at pH 5, decreased from 78.4% of the initial at day 0 to not detectable by day 3 at pH 7, decreased from 52.3% of the initial at day 0 to not detectable by 24 hours at pH 9, and decreased from 58.0% of the initial at day 0 to not detectable by 24 hours in seawater. At pH 5, the major transformation product detected was CL 322,250 with a maximum concentration of 73.9% of the applied amount observed at the day 30. At pH 7, the major transformation products detected were CL 322,250 and Unknown B with maximum concentrations of 72.4% and 29.6% of the applied observed on the 7th and 30th days of incubation, respectively. At pH 9, the major transformation product detected was CL 322,250, with a maximum concentration of 96.9% of the applied amount observed on the 7th day of incubation. ln seawater, the major transformation product detected was CL 322,250 with a maximum concentration of 96.3% of the applied amount observed 24 hours after incubation. The minor transformation products detected at pH 5 were CL 325,195; Unknown C; and Unknown D formed at maximum concentrations of 2.9, 2.1, and 2.3% of the applied, respectively. The minor transformation products detected at pH 7 were CL 325,195; Unknown A; Unknown C; and Unknown D formed at maximum concentration of 1.4, 7.2, 1.5, and 1.9% of the applied, respectively. The minor transformation products detected at pH 9 were CL 325,195; Unknown A; Unknown C; Unknown D; and Unknown F formed at maximum concentrations of 2.2, 1.2, 1.0, 1,9, and 1.4% of the applied, respectively. The minor transformation products detected in seawater were CL 325,195; Unknown A; Unknown C; Unknown D; and Unknown F formed at maximum concentrations of 2.7, 1.1, 1.0, 1.6, and 1.7% of the applied, respectively. Volatiles were not formed.

The hydrolytic half-lives of [^{14}C]-R107894 in pH 5, pH 7, pH 9 and seawater at 25EC were calculated as 15 days, and 8, 2, and 3 hours, respectively. The corresponding values for [^{14}C]-R107894 incubated at 10EC were 168 days, and 69, 12, and 15 hours, respectively. [^{14}C]-R107894 was found to be hydrolytically unstable under the conditions of the test. Rapid hydrolysis was observed in pH 7, pH 9, and seawater incubated at 25EC, in comparison with that observed at pH 5. While hydrolysis was slower at 10EC, [^{14}C]-R107894 would still be classified as unstable.

ln the supplemental study (MRlD No. 456739-09), solutions of [^{14}C]-R107894 in aqueous buffer (pH 7 and pH 9) and seawater were incubated at 10EC and 25EC for up to 96 hours to investigate the hydrolytic stability of R107894. Two hydrolysis products were detected together with two unknowns (A and B) which were only present in the pH 7 samples. The hydrolysis products (CL 322,250 and CL 325,195) were confirmed as being present in all the

samples analyzed and the unknowns were identified as isomers of a condensation reaction between Tris(tris(hydroxymethyl)amino methane, from the pH 7 buffer) and CL 322,250. The unknowns were not true hydrolysis products from the incubation, but artifacts arising from the buffer used with the pH 7 samples.

2. Photodegradation in Water (Guideline No. OPPTS 161-2, Waived)

The Agency has waived data requirements for the photodegradation of ECONEA™ Technical. The active ingredient 1H Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl-5-(trifluoromethyl) is hydrolytically unstable and rapidly degrades. Photolysis studies were, therefore, not required.

3. Anaerobic Aquatic Metabolism (Guideline No. OPPTS 162-3, MRID No. 456739-10)

This anaerobic aquatic metabolism study was reviewed by the Agency and found to be acceptable for the active ingredient 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as R107894. The anaerobic aquatic metabolism data requirements for ECONEA™ Technical have been satisfied.

The anaerobic biotransformation of [^{14}C]-R107894 was studied in both a freshwater-sediment and a marine-sediment test system from Scotland for 52 weeks in the dark at 21°C. [^{14}C]-R107894 was applied at the rate of 69 μg/L to the surface of the water in each sample. The sediment/water ratio used was 15g/150mL. The test system consisted of borosilicate glass cylinders attached with traps for the collection of CO_2 and volatile organic compounds. Samples were analysed at 0, 3, 7, 14 and 30 days and 8, 13, 17, 26, 39, and 52 weeks of incubation. Surface water was separated from the sediment by decanting and transferred into separate amberlite jars. The water samples were not extracted and the sediment samples were extracted with acetonitrile twice with approximately 50 mL. [^{14}C]-R107894 residues were analysed by thin layer chromatography (TLC) (using a silica gel 60F_{254} TLC plate and developed in toluene:acetone:methanol:acetic acid) and high performance liquid chromatography (HPLC) (using a Hewlett-Packard 1050 series). Identification of the transformation products was done by co-chromatography.

The test conditions outlined in the study protocol were maintained throughout the study. The mean total recovery of radiolabelled material after 52 weeks was 100.4±4.8% and 96.97±2.2% of the applied in the freshwater-sediment system and the marine-sediment system, respectively. The mean total recovery of radiolabelled material in the surface water and sediment of the freshwater test system was 26.30±1.1% and 22.91±0.9% of the applied amount, respectively. In the marine test system, the mean total recovery of radiolabelled material in the surface water and sediment was 57.68±0.2% and 22.46±1.2% of the applied amount, respectively.

In the fresh water test system, extractable [^{14}C]-residues in sediment decreased from a high of 62.80% at day 7 to 22.91% of the applied amount at the end of incubation period. Non-extractable [^{14}C]-residues in sediment increased from a low of 0.30% at day 3 to 50.96% of the applied amount at the end of the incubation period. In the marine test system, extractable [^{14}C]-residues in sediment decreased from a high of 32.29% at day 14 to 22.46% of the applied amount at the end of incubation period. Non-extractable [^{14}C]-residues in sediment increased from a low of 1.01% at day 3 to 16.52% of the applied amount at the end of incubation period. At the end of the study, 0.11% and 0.02% of the recovered radioactivity was present as CO_2 and volatile organic compounds, respectively, in the marine test system. In the fresh water test system, 0.04% and 0.02% of the recovered radioactivity was present as CO_2 and volatile organic compounds, respectively.

In the fresh water test system, the concentration of R107894 in surface water and sediment decreased from 90.19% at day 0 to 1.80% of the applied amount at study termination. In the marine test system, the concentration of R107894 in surface water and sediment decreased from 92.36% to 0.06% of the applied amount at study termination.

The major transformation products of both the fresh water system and the marine system detected by HPLC analysis in water and sediment were CL 322,250 and CL 325,195. Maximum and minimum concentrations in the water of the freshwater test system were 44.10% and 2.56% of the applied amount, for CL 322,250, while CL 325,195 was reported to be below the detection limit throughout the incubation period. Maximum and minimum concentrations in the water of the marine test system were 60.34% and 1.99% of the applied amount for CL 322,250, and 6.64% and below the detection limit for CL 325,195. Maximum and minimum concentrations in the sediment of the freshwater test system were 10.05% and 4.62% of the applied amount for CL 322,250, and 1.29% and 1.16% of the applied amount for CL 325,195. Maximum and minimum concentrations in the sediment of the marine test system were 16.35% and 2.38% of the applied amount, for CL 322,250, and 1.39% and 0.52% of the applied amount for CL 325,195.

The 1st order 50% decline time (DT50) for the freshwater test system was 10 days and the 90% decline time (DT90) was 113 days. For the marine test system, the 1.5 order DT50 was 0.03 days and the DT90 was 0.83 days. The rates of degradation were estimated by fitting the data to the Timmes, Frehse, and Laska model. Degradation was very rapid in the marine test system and the degradation rates of R107894 in each of the compartments could not be estimated with any degree of accuracy due to the variability in the total levels of radioactivity in each of the compartments over the incubation period.

4. Aerobic Aquatic Metabolism (Guideline No. OPPTS 162-4, MRID Nos. 456739-11 and 456739-12)

This aerobic aquatic metabolism study was reviewed by the Agency and found to be acceptable for the active ingredient 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as R107894. The aerobic aquatic metabolism data requirements for

ECONEA™ Technical have been satisfied.

In the main part of the study (MRID No. 456739-11), the biotransformation of radiolabelled [^{14}C]-R107894 was studied in a freshwater/sediment system (water pH 6.5, silt loam, organic carbon 2.5%) and a marine water/sediment system (water pH 8.04, sandy loam, pH 7.53, organic carbon 0.8%) collected from Bogton Loch and Seaby Bay in Scotland. The experiment was performed for 30 days under aerobic conditions in the dark at 21^{0}C. Radiolabelled R107894 was applied at the rate of 0.5 mg/L. The test system consisted of borosilicate glass cylinders (previously silanised; 15.9 cm^2 cross-sectional area) as the incubation vessel and included a series of three traps for trapping non-specific [^{14}C]-organic volatiles and liberated $^{14}CO_2$. Samples were collected at 0, 2 hours, and 1, 3, 7, 15, and 30 days of incubation. The water samples were not extracted. The sediment samples were extracted twice with 50 ml of acetonitrile and then shaken for 1 hour, followed by centrifugation for 15 minutes. Quantification and identification of the [^{14}C]-R107894 residues was performed using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

For the silt loam (freshwater) test system, the mean overall recovery of radiolabelled material was 93.8 ± 5.2% of the applied amount. For the loamy sand (marine water) test system, the mean overall recovery of radiolabelled material was 95.5 ± 4.4% of the applied amount.

The concentration of the parent compound in freshwater immediately after the application showed a mean of 51.2% of the applied amount and had dropped below the detection limit by the end of the study period (Day 30). The concentration of the parent compound in the silt loam (freshwater) sediment decreased from a mean of 36.3% of the applied amount at Day 0 to a mean of 16.4% of the applied amount at the study termination. The concentration of the parent compound in marine water decreased from a mean of 77.2% of the applied amount at Day 0 to below the detection limit by Day 15 of the study. The concentration of the parent compound in loamy sand (marine) sediment decreased from a mean of 18.05% of the applied amount at Day 0 to a mean of 4.04% by Day 7.

The DT50 and DT90 values were estimated by visual inspection of the data by the Registrant. The DT50 for [^{14}C]-R107894 in the freshwater silt loam system was estimated as being between 3 and 7 days and the DT90 was estimated as being just over 30 days. In the marine water loamy sand test system, the DT50 and DT90 were estimated as being less than 1 day and approximately 7 days, respectively. The two major transformation products were CL 322,250 and Unknown B (a supplementary study tentatively identified this component as debrominated CL 322,250). There were four minor transformation products. These minor transformation products were referred to as CL 325,195, Unknown A, Unknown C, and Unknown D.

For the silt loam sediments, extractable ^{14}C-residues decreased from a mean of 38.1% of the applied amount at Day 0 to a mean of 26.2% of the applied amount at study termination. Non-extractable [^{14}C]-residues increased from a mean of 1.82% of the applied amount at Day 0

to a mean of 36.43% of the applied amount at the end of incubation period. For the loam sand sediments, extractable ^{14}C-residues increased from a mean of 21.4% of the applied amount at Day 0 to a mean of 33.7% of the applied amount at study termination. Non-extractable [^{14}C]-residues increased from a mean of 0.275% of the applied amount at Day 0 to a mean of 6.54% of the applied amount at the end of the incubation period.

For the freshwater silt loam sediment system, there were no detectable levels of radioactivity present as CO_2 or volatile compounds at the end of the study. For the marine water loamy sand sediment system, a mean of 0.02% of the recovered radioactivity was present as CO_2. Volatile compounds were not detectable.

A supplemental study (MRID No. 456739-12) was also performed. One of the major transformation products from the main study (MRID 456739-11) was labeled as Unknown B and it had a retention time of approximately 26 minutes following the analysis of samples generated by the loamy sand (marine) test system. For this supplemental study, two water samples from Day 30 were taken and concentrated by solid phase extraction. The concentrated samples were analyzed by negative ion electrospray liquid chromatography mass spectrometry in addition to radiochemical detection. Two peaks were identified in the radiochromatogram during the supplementary study. The latter of these was confirmed as CL 322,250 by comparison of retention time, full scan spectrum and daughter spectrum to those obtained following the analysis of authentic CL 322,250. The first peak (Unknown B) was tentatively postulated as debrominated CL 322,250 based on comparison of retention times, spectra and daughter spectra for this peak and the CL 322,250 reference standard.

5. Adsorption/Desorption (Guideline No. OPPTS 163-1, MRID No. 456739-13)

This adsorption/desorption study was reviewed by the Agency and found acceptable for the active ingredient 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as R107894. The adsorption/desorption data requirements for ECONEA™ Technical have been fulfilled.

The adsorption/desorption characteristics of [^{14}C]-R107894 were studied in two freshwater soils, sandy loam and silt loam, and two marine soils, sand and loam, from Scotland in a batch equilibrium experiment. The adsorption phase of the study was carried out by equilibrating air-dried/fresh soil with [^{14}C]-R107894 at 0, 54, 109, 268, and 518 ng/g soil for sandy loam and silt loam and at 0, 47, 96, 242, and 484 ng/g soil for sand and loam in the dark at 10 ± 2 °C for 4 hrs for all the soils but sand, which was equilibrated for 8 hrs. The equilibrating solution used was 0.01M $CaCl_2$ or seawater, with a soil/solution ratio of 2g/10g. The desorption phase of the study was carried out by adding a weight of 0.01M calcium chloride or seawater, approximately equal to that removed as supematant, to each soil type. The tubes were shaken and analyzed as in the adsorption phase.

The supematant solution after adsorption and desorption was separated by centrifugation.

The supernatant was not extracted. [^{14}C]-R107894 residues were analysed by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). HPLC analysis was carried out using a Hewlett-Packard 1050 series HPLC equipped with an autosampler, u.v. detector and a solvent programmer, connected to an Inertsil Phenyl guard and HPLC column (1 cm and 25 cm x 4.6 mm; 5 μm; Hichrom) and a Packard Flo-One A-100 Series radioactivity monitor. Aliquots of each sample were also submitted to TLC using a silica get 60_{F254} TLC plate and developed in toluene:acetone:methanol:acetic acid. The adsorption parameters were calculated using the Freudlich adsorption isotherm.

The stability of the test material at 10 ± 2°C in 0.01M calcium chloride and seawater was determined by HPLC. Under the test conditions, [^{14}C]-R107894 was found to be unstable. However, the study author found that these test conditions best reflect those that the test material will enter in the environment. The mass balance at the end of the adsorption phase of the study was 90.99 ± 2.1, 89.45 ± 3.4, 100.5 ± 6.9, and 103.8 ± 2.0% of the applied amount in the sandy loam, silt loam, sand, and loam soils, respectively. The mass balance at the end of desorption phase was 91.50 ± 1.1, 93.70 ± 4.9, 104.3 ± 7.6, and 99.66 ± 0.9% of the applied amount in sandy loam, silt loam, sand, and loam soils, respectively.

After 4 hr of equilibration for sandy loam, silt loam, loam and 8 hr of equilibration for sand, an average of 98.89, 98.38, 97.48, and 83.18% of the applied amount was adsorbed, respectively. Average adsorption K_d values were 450, 335, 26, and 196 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. The average adsorption K_{oc} values were 20440, 16733, 3582, and 5588 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. K_f values were 446, 349, 22, and 183 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. At the end of the desorption phase, 0.84, 0.88, 9.62, and 1.63% of the adsorbed ^{14}C was desorbed in the sandy loam, silt loam, sand, and loam soils, respectively. Average desorption K_d values were 599, 568, 40, and 299 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. The average desorption K_{oc} values were 27229, 28353, 5658, and 8543 ml/g for sandy loam, silt loam, sand, and loam soils, respectively. Desorption K_d and K_{oc} values were higher than those obtained for adsorption.

6. Bioaccumulation in Fish (Guideline No. OPPTS 165-4, Agency Estimated BCF) (No MRID Number))

The Agency estimated the bioconcentration of the ECONEA™ Technical degradate CL 322,250 in freshwater and saltwater fish based on the log octanol/water partition coefficient. Using equations presented in the OECD TG 305 Guideline, estimated bioconcentration factors (BCFs) of 11X (pH6) and 3X (pH8) were predicted in freshwater and saltwater fish, respectively, for the bluegill sunfish.

7. Special Leaching Study (Guideline ASTM Standard Test Method D 5108-90, MRID No. 456732-01)

This leaching study was reviewed by the Agency and found to be acceptable for the active ingredient 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluromethyl), also known as AF028. The leaching data requirements for ECONEA™ Technical have been satisfied.

The leach rate determination of Sigma Nexxium 20 paint was studied using the ASTM D 5108-90 Method: *Standard Test Method for Organotin Release Rates of Antifouling Coating Systems in Sea Water*, specifically designed for antifoulants. The study was conducted to determine the rate at which two active ingredients, one of which is AF028, are released from Sigma Nexxium 20 Paint. The study was conducted in synthetic seawater prepared at 25 ± 2°C, using high performance liquid chromatography (HPLC). The salinity of the synthetic seawater, was maintained between 30 and 35 ppt and a pH of 7.8 to 8.2. The study of leach rate measurement was conducted for 45 days. Cylinders were put in the holding tank (food-grade polyolefin) of 100 L capacity. Synthetic seawater was continuously pumped through the tank, an activated carbon filter and a chelating resin filter at 5L/min. Leach rates were measured by exposing the cylinders to 1500 mL of synthetic seawater and rotating the cylinders for 60 minutes at 60 ± 5 rpm. The leach rates were measured on days 1, 3, 7, 10, 14, 21, 24, 28, 31, 38, 42 and 45. Samples of the leached Sigma Nexxium 20 Antifouling paint were collected and analyzed for AFO28 by HPLC.

The pseudo steady state leach rate for AFO28 was attained in 28 days. The average leach rate of AFO28 between day 28 and 45 was 8.00 Φg/cm^2/day. The average cumulative release of AFO28 was 12.9 Φg/cm^2 through day 1 and 454 Φg/cm^2 through day 45. Sigma Nexxium 20 paint was applied to polycarbonate cylinders with measurements of 2.5 inches in diameter (cylinder length not reported). The area of paint applied on the cylinder was 200 cm^2. Film thickness was at least 0.004 inches.

8. Additional Analyses Performed by U.S. EPA (EFED) (Power Point Presentation)

The Agency (EFED) also performed regression analyses to estimate the half-lives in freshwater and seawater for ECONEA™ Technical (parent) and its degradate CL 322,250. The analyses were based on the information provided in the study reports submitted to the Agency to fulfill the hydrolysis (MRID Nos. 456739-08 and 456739-09), anaerobic aquatic metabolism (MRID No. 456739-10, aerobic aquatic metabolism (MRID Nos. 456739-11 and 456739-12), and adsorption/desorption (MRID No. 456739-13) data requirements for the active ingredient, 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-trifluoromethyl.

The estimated half-lives and adsorption/desorption of the parent and degradate are presented in the following tables:

Table 1. Hydrolysis Half-Lives in Freshwater and Seawater at 10 and 25°C (days)

	ECONEA (parent)

pH	10°C	25°C
5	177	15
7	2.8	0.33
9	0.56	0.1
Seawater (pH 8)	0.7	0.1

Note: 322,250 did not degrade in the hydrolysis study at any pH or temperature

Table 2. Anaerobic Aquatic Metabolism (representing sediment)

Compound	Half-life (days	Comments
ECONEA (parent) Freshwater	29	322,250 was primarily found in the water phase. Debrominated 322,250 did not decline and was found primarily in the water phase
322,250 Freshwater	31	
ECONEA (parent) Marine	0.68	
322,250 Marine	22	

Table 3. Acrobic Aquatic Metabolism (representing water column)

Compound	Half-life (days	Comments
ECONEA (parent) Freshwater	12	322,250 was primarily found in water phase in both systems. No observed formation of debrominated 322,250 in freshwater system Debrominated 322,250 did not decline in saltwater system and was found primarily in the water phase
322,250 Freshwater	Stable	
ECONEA (parent) Marine	0.62	
322,250 Marine	288	

Table 4. Adsorption of Parent ECONEA

System	Adsorption coefficients Kf (ml/g)	Adsorption coefficients K_{oc} (ml/g)	Comments
Marine Freshwater	22-183 349-446	3143-5229 17450-20273	No correlation with clay, organic matter, or pH.

Table 5. Desorption of Parent ECONEA

System	Desorption coefficients Kf (ml/g)	Desorption coefficients K_{oc} (ml/g)	Comments
Marine Freshwater	32-236 463-480	4571-6743 21818-23150	No correlation with clay, organic matter, or pH.

Table 6. Adsorption of Degradate CL 322,250

System	Adsorption coefficients Kf (ml/g)	Adsorption coefficients K_{oc} (ml/g)	Comments
Marine Freshwater	14-119 189-357	2000-3400 8591-17850	Correlation with clay and pH.

Table 7. Desorption of Degradate CL 322,250

System	Desorption coefficients Kf (ml/g)	Desorption coefficients K_{oc} (ml/g)	Comments
Marine Freshwater	30-283 1260-1685	4310-8084 57256-84250	Correlation with clay and pH.

The Agency concluded that:

- Parent degrades to 322,250 (parent minus fluorines and remaining carbon hydrated)
- 322,250 further degrades by losing a Bromine (debrominated 322,250)
- Debrominated 322,250 is only formed under anaerobic conditions or in saltwater

- Metabolism studies show 322,250 and debrominated 322,250 to be primarily in water phase
- However, mobility data on 322,250 show partitioning to sediment
- No mobility data for debrominated 322,250.

Data Gap: See Table below.

Environmental Fate Data Requirements for Econea™ Technical			
OPP Guideline	**Data Requirement**	**MRID No.**	**Data Requirement Status**
161-1	Hydrolysis	456739-08 456739-09	Satisfied
161-2	Photodegradation in Water	None	Waived
162-3	Anaerobic Aquatic Metabolism	456739-10	Satisfied
162-4	Aerobic Aquatic Metabolism	456739-11 456739-12	Satisfied
163-1	Adsorption/Desorption	456739-13	Satisfied
OECD 305	Bioaccumulation in Fish	None	Estimated

ASTM D5108-90	Special Leaching Study	476732-01	Satisfied

BIBLIOGRAPHY

MRID	CITATION
456732-01	Sinning, D.J. (2002) Leach Rate Determination of Sigma Nexxium 20 Paint Containing Sea Nine™ 211 and AF028 Antifoulings. Unpublished study prepared by Case Consulting Laboratories, Inc., New Jersey.
456739-08	Mackie, J.A. (1997) Determination of the Hydrolytic Stability of [^{14}C]-R107894: Report Number 15348. Unpublished study prepared by Inveresk Research, Scotland.
456739-09	Milligan, F.M.; Williams, S.G.P.; McGuire, G.M. (1997) Identification of Hydroytic Degradation Products of [^{14}C]-R107894: Report Number 15365: Supplement to MRID 456739-08. Unpublished study prepared by Inveresk Research, Scotland.
456739-10	Mackie, J.A. (1999) The Anaerobic Degradation of [^{14}C]-R107894 in Two Water/Sediment Systems: Report Number 17832. Unpublished study prepared by Inveresk Research, Scotland.
456739-11	Mackie, J.A. (1999) The Aerobic Degradation of [^{14}C]-R107894 in Two Water/Sediment Systems: Report Number 16787. Unpublished study prepared by Inveresk Research, Scotland.
456739-12	Unknown author(s) (1999) Identification of Unknown Component Present in a Day 30 Surface Water Following Application of [^{14}C]-R107894 to Loamy Sand Sediment: Report Number 17802: Supplement to MRID 456739-11. Unpublished study prepared by Inveresk Research, Scotland.
456739-13	Mackie, J.A. (1998) Adsorption/Desorption of [^{14}C]-R107894 in Sediments: Report Number 390723. Unpublished study prepared by Inveresk Research, Scotland.
None	U.S. EPA (2004) ECONEA Fate and Transport Properties. Power Point presentation presented April 13, 2004, by Jim Breithaupt, Environmental Fate and Effects Division (EFED).

Appendix B: Estimated Environmental Concentrations for ECONEA™ Antifoulant Agent

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

November 15, 2006

EPA United States Environmental Protection Agency Office of Pesticide Programs

MEMORANDUM

SUBJECT: Estimated Environmental Concentrations (EECs) for ECONEA ™ Antifouland Agent

From: Siroos Mostaghimi, Senior Scientist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

James Breithaupt, Agronomist
Environmental Risk Branch II
Environmental fate and Effects Division (7507P)

To: Marshall Swindell, PM 33
Regulatory Management Branch I (RMBI)
Antimicrobials Division (7510P)

Thru: Norm Cook, Chief
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

DP Barcode: 330451

Pesticide Chemical No.: 119093
Registrant: Janssen Pharmacetuica Inc.

The following report contains a summary of the results from modeling data which were submitted by the Janssen Pharmacetuica Inc. in a submission titled "Environmental and Ecological Risk Assessment of ECONEA Antifoulant Agent (MRID# 468466-03)" . The inputs used for running MAM-PEC (Marine Antifoulant Model to predict Environmental Concentrations) and, EFDC (Environmental Fluid Dynamic Code), appear correct and the data reported from the runs are acceptable. The inputs for the TRIM2D (Tidal Residual Inter-tidal Mudflat) appear correct; however, the outputs from this model run could not be verified independently because of the licensing issues and the lack of availability of TRIM2D algorithms to the public.

Discussion and Conclusion

RASSB concludes that the data submitted by the registrant for modeling runs of MAMPEC and FEDC are acceptable and appear to be scientifically sound. However, the data from the TRIM2D could not be verified.

MAM-PEC is used as an assessment tool for antifoulant risk assessments in Europe. MAM-PEC was developed by the Institute of Environmental Studies/IVM and Delft Hydraulics for the European Paint Makers Association (CEPE) for conducting risk assessments for antifouling agents. The model provides prediction of environmental concentrations of antifouling products in six generalized "typical" marine environments (commercial harbor, estuarine harbor, marina, marina poorly flushed, open sea, and shipping lane).

FEDC is a multifunctional surface water modeling system, which includes hydrodynamic, sediment-contaminant, and eutrophication components. The EFDC model is capable of 1, 2, and 3-D spatial resolution. The model uses a curvilinear-orthogonal horizontal grid and a sigma terrain following vertical grid. The EFDC model can represent the transport and fate of an arbitrary number of contaminants, including metals and hydrophobic organics, sorbed to any of the sediment classes and dissolved and particulate organic carbon using a three-phase equilibrium partitioning formulation. The public domain EFDC program was originally developed at the Virginia Institute of Marine Science and is currently maintained by Tetra Tech, Inc. with support from the US EPA.

TRIM2D is a 2-dimensional, depth-averaged, finite-difference hydrodynamic model for simulating inland water flows governed by tidal, wind and riverine inputs. The model uses a high-resolution uniform grid to solve the incompressible Navier-Stokes equations. Simulation output includes water velocities, water surface elevations, salinity profiles, and the distribution of any released contaminants. The TRIM2D software was developed by the Space and Naval Warfare System Center San Diego (SSC SD), within the Department of the Navy, in collaboration with the U.S. Geological Survey (USGS). The algorithm for this model is not available to the public.

The active ingredient in ECONEA™ is R107894 (1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-

chlorophenyl)-5-trifluoromethyl), also known as CL303268. R107894 degrades rapidly to three metabolites (CL322250, CL322348 and CL325195).

R107894 breaks down rapidly in the environment. Degradation from aqueous hydrolysis has been reported to occur with half-lives of 3 and 15 hours in seawater (at temperatures of 25° and 10° C, respectively), and 2 and 12 hours in freshwater at pH 7 (25° and 10°C, respectively). Half-lives of 2 to 4 days in water have been reported in marine and freshwater aerobic aquatic metabolism studies. Half-lives in sediment or full test system were longer in those studies (31 and 13 days, respectively).

Degradation products include CL322250 and CL325195. CL322250 breaks down further to form CL322248. Maximum formation (percent of R107894) observed in marine aerobic aquatic metabolism studies have been 70, 76, and 7 percent for CL322250, CL322248, and CL325195, respectively.

The study submitted by the registrant focuses on CL322250 and CL322248 based on their expected respective rates of formation, persistence, toxicity, and potential for toxicological effects in the environment. R107894 is not addressed because of its rapid degradation in the environment and low potential for bioaccumulation. CL322195 is not addressed based on its relatively low rate of formation and low toxicity to test species.

Model simulations were used to estimate the concentrations of the CL322250 and CL322248 in five harbor system in the United States. The systems modeled, models used and the rationale for use of the models are presented in the Table 1.

Table1. Models used for estimating environmental concentrations of ECONEA ™ in different systems.

System	Model	Rationale
Commercial, Estuarine, Marina, Marina Poorly Flushed, Shipping Lane, and Open Sea	MAM-PEC	Screening level assessment using standard environments developed for the European Union.
Barbours Cut – Houston	MAM-PEC	Screening level representation of harbor system developed for this study.
Baltimore Harbor	MAM-PEC	Screening level representation of harbor system developed for this study.
Norfolk Harbor/James River	EFDC	Detailed representation of harbor system previously setup by VIMS.

Port of New Orleans, lower Mississippi River	EFDC	Detailed representation of harbor system developed for this study.
San Diego Bay	TRIM2D	Detailed representation of harbor system previously developed by SSC SD.

Estimated Environmental Concentrations (EECs):

The estimated environmental concentrations for CL322250 and CL322248 from MAM-PEC runs in Baltimore and Barbarous Point Houston are shown in table 2. Both maximum and average concentration in water column and sediments are presented in this table.

Table2. Maximum and Average concentrations of CL322250 and CL322248 in Baltimore harbor and Barbours Point Houston estimated by MAM-PEC model.

Chemical	Statistics	Location			
		Baltimore		Barbours Point Houston	
		Water µg/l	Sediment (µg/g dw)	Water µg/l	Sediment (µg/g dw)
CL322250	Maximum	0.041	7.77E-5	0.448	8.44E-4
	Average	0.024	4.51E-5	0.335	6.32E-4
CL322248	Maximum	0.037	1.54E-4	0.406	1.66E-3
	Average	0.022	8.92E-5	0.304	1.24E-3

The EECs for CL322250 and CL322248 from the TRIM2D model run in San Diego Harbor are presented in Table 3. The chemical partitioning to sediments were not predicted by TRIM2D, therefore only concentrations in water are shown in this table.

Table 3 Maximum and Average concentrations of CL322250 and CL322248 in San Diego Harbor estimated by TRIMD2 model

Chemical	Statistics	Location
		San Diego harbor
		Water (µg/l)
CL322250	Maximum	3.840
	Average	1.816
CL322248	Maximum	4.174
	Average	2.173

The concentrations for CL322250 and CL322248 from the EFDC model results in Norfolk Harbor and Mississippi River are presented in Table 4. Both maximum and average concentration in water column and sediments are presented in this table.

Table4. Maximum and Average concentrations of CL322250 and CL322248 in Norfolk Harbor and Mississippi River estimated by EFDC model.

Chemical	Statistics	Location			
		Norfolk		Mississippi River	
		Water µg/l	Sediment (µg/g dw)	Water µg/l	Sediment (µg/g dw)
CL322250	Maximum	0.760	4.87E-3	0.233	<1.0E-10
	Average	0.180	4.05E-4	0.019	<1.0E-10
CL322248	Maximum	0.742	3.92E-4	0.211	<1.0E-9
	Average	0.0.188	7.85E-5	0.017	<1.0E-9

The estimated concentrations from the Mississippi River should be used for the dietary exposure assessment. The maximum concentrations should be used for short term and the average concentrations should be used chronic dietary assessment.

The data from MAP-PEC result for the Barbours Point Houston in water should be used for the ecological risk assessment. It should be noted that the highest concentrations were reported in San Diego Harbor by the TRIMD2 model. However, because of the lack of enough information for the TRIMD2 model the data from this model could not be verified independently.

File: C:\Myfiles\2006 Reports/ ECONEA Modeling\EECs for ECONEA

CC: Siroos Mostaghimi/RASSB
RASSB Chemical Files

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

JANUARY 19, 2006

MEMORANDUM

Subject: Review of three algal toxicity studies, two studies using *Skeletonema costatum,* and one using *Pseudokirchneriella subcapitata* as test organisms. Two studies were submitted to support the proposed registration of Econea Technical and the third study for a degradate (CL322,250). (DP Barcode 321453; Decision# 220066; PC Code 119093)

From: David C. Bays, Risk Assessment and Science Support Branch (RASSB), Antimicrobials Division (7510C) 1/19/06

To: Marshall Swindell, Product Manager #34, Antimicrobials Division (7510C)

Thru: Rick Petrie, Team 3 Leader, RASSB, AD (7510C) Mark for

Kathryn Montague, Acting Team 1 Leader, RASSB, AD (7510C) Mark for

And

Norm Cook, Branch Chief, RASSB, AD (7510C) Norman A. Cook

RSSAB has completed the review of three algal toxicity studies (MRIDs 46596006, 46596014, and 46619901) with Econea Technical and a major degradate (CL322,250) as the test chemicals. The proposed use pattern for Econea Technical is as an antifoulant paint product. The first study, MRID 46596006, tested the toxicity of Econea Technical against the Freshwater Green Alga *Pseudokirchneriella subcapitata.* The chemical was found to be very highly toxic to the green alga under static conditions. The 96-hour EC_{50} was determined to be 0.011 mg a.i./L, with 95% confidence intervals of 0.0105 to 0.011 mg a.i./L. The NOEC was determined to be 0.0068 mg a.i./L. Due to some omissions in the study report and some deviations in protocol, the study will be considered supplemental and not repairable. These omissions are as follows: sterilization/cleaning practices, water solubility, and physical/chemical properties of the chemical, including saturation concentration. A major deviation from protocol was reported (the starting number of algal cells was too low [1,000 instead of 10,000]). A

second protocol deviation was also reported (the lowest concentration of the range-finding test was not at the detection limit). Since the low starting number of algal cells cannot be changed, the study is not repairable and cannot be upgraded to core.

The second study, MRID 46596014, tested the toxicity of a major degradate of Econea to the marine diatom *Skeletonema costatum*. This degradate was found to be moderately toxic to the marine diatom under static conditions. The 96-hour EC_{50} was determined to be 0.66 mg a.i./L, with 95% confidence intervals of 0.60 to 0.70 mg a.i./L. The NOEC was determined to be 0.50 mg.a.i. /L. Due to some omissions in the study report, the study will be considered supplemental. These omissions are as follows: sterilization/cleaning practices, water solubility, and physical/chemical properties of the chemical, including saturation concentration. If the registrant provides the missing information, then the study may be upgraded to core.

The third study, MRID 46619901, tested the toxicity of Econea to the marine diatom, *Skeletomema costatum*. This chemical was found to be very highly toxic to the marine diatom under static conditions. The 96-hour EC_{50} was determined to be 0.0027 mg a.i./L, with 95% confidence intervals of 0.0026 to 0.0029 mg a.i./L. The NOEC was determined to be 0.0015 mg a.i./L. The study was scientifically sound and will be considered core.

If you have any questions on the above, please contact David Bays at 703-605-0216.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

3/20/06
~~FEBURARY 17, 2006~~

MEMORANDUM

Subject: Review of four aquatic toxicity studies, using *Oncorhynchus mykiss, Lepomis macrochirus, and Daphnia magna* (both acute and chronic testing) as test organisms, submitted to support the proposed registration of Econea Technical. (DP Barcode 325938; Decision# 220066; PC Code 119093)

From: David C. Bays, Risk Assessment and Science Support Branch (RSSAB), Antimicrobials Division (7510W) 2/17/06

To: Marshall Swindell, Product Manager #33, Antimicrobials Division (7510W)

Thru: Rick Petrie, Team 3 Leader, RASSB, AD

Kay Montague, Acting Team 2 Leader, RASSB, AD for 3/20/06
And

Norm Cook , Branch Chief, RASSB, AD 3/20/06

RSSAB has completed the review of four aquatic toxicity studies (MRIDs 46596001, 46596002, 46596003 and 46596004) with Econea Technical as the test chemical. Econea Technical is used as an anti-foulant paint product. The first study was an aquatic invertebrate acute toxicity test using Freshwater Daphnids, *Daphnia magna*, as the test organism (OPPTS 850.1010). There were some guideline deviations identified by the reviewer that may have affected the results of the study (see DER for MRID 46596001). Therefore, the study is classified as supplemental but may be upgraded if the registrant clarifies low recoveries when measuring concentrations. As reported, the results were as follows: 48-hour EC_{50} was 1.5 µg a.i./L (95% C.I. = 1.2-1.9 µg a.i./L) and the NOEC was 0.32 µg a.i./L, which indicates that Econea Technical is acutely highly toxic to freshwater daphnids.

The second study (MRID 46596002) was a fish acute toxicity test using Rainbow Trout, *Oncorhnchus mykiss*, as the test organism (OPPTS 850.1735). There were some guideline deviations identified by the reviewer, but these were minor in nature and did not affect the results of the study (see DER for MRID 46596002). Therefore, the study is classified as core and can be used in a risk assessment. As

reported, the results were as follows: 96-hour LC50 was 1.3 µg a.i./L (95% C.I. = 0.68-2.1 µg a.i./L) and the NOEC was 0.68 µg a.i./L, which indicates that Econea Technical is highly toxic to rainbow trout.

The third study (MRID 46596003) was a fish acute toxicity test using Bluegill Sunfish, *Lepomis macrochirus*, as the test organism (OPPTS 850.1075). There were some guideline deviations identified by the reviewer, but these were minor in nature and did not affect the results of the study (see DER for MRID 46596003). Therefore, the study is classified as core and its results can be used in a risk assessment. As reported, the results were as follows: 96-hour LC50 was 3.2 µg a.i./L (95% C.I. = 2.8-3.7 µg a.i./L) and the 96-hour NOEC was 1.3 µg a.i./L, which indicates that Econea Technical is highly toxic to bluegill sunfish.

The fourth study (MRID 46596004) was a daphnid chronic toxicity test using Freshwater Daphnids, *Daphnia magna*, as the test organism (OPPTS 850.1300). Pre-test culture conditions, including presence of ephippia, were not reported. Presence of ephippia invalidates the test; additionally, information on aeration was not provided. This information must be submitted in order to upgrade this study to acceptable. If suitable information regarding pre-test culture conditions and aeration is submitted, study may be upgraded.

If you have any questions on the above, please contact David Bays at 703-605-0216.

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE ACUTE TOXICITY TEST, FRESHWATER DAPHNIDS
GUIDELINE OPPTS 850.1010

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl) (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** R107894 **Purity:** 94.6%
   Lot or Batch No.: AC12649-8

3. **CITATION**

Authors:	Mark A. Cafarella
Title:	R107894 – Acute Toxicity to Water Fleas (*Daphnia magna*) Under Flow-Through Conditions
Study Completion Date:	June 28, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N. V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6141
MRID No.:	465960-01

4. **REVIEWED BY:**

   Signature: **Date: 2/17/06**
   David Bays, Microbiologist, RASSB, AD (7510C)

5. **APPROVED BY:**

   Signature: **Date: ~~2/17/06~~**
   Rick Petrie, Team 3 Leader, RASSB, AD (7510C)
   Kay Montague, Acting Team 2 Leader, RASSB, AD (7510C) for 3/20/06

6. **STUDY PARAMETERS**

   Scientific Name of Test Organism: *Daphnia magna*
   Age of Test Organism: 24 hours
   Definitive Test Duration: 48 hours
   Study Method: Flow-through
   Type of Concentrations: Nominal and mean-measured

7. **CONCLUSIONS**

Results Synopsis:

48-hour EC_{50}:	1.5 µg a.i./L	95% C.I.:	1.2-1.9 µg a.i./L
NOEC:	0.32 µg a.i./L		

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: Appears to be a scientifically sound study, but had some guideline deviations that may have affected the results of the study.

C. Repairability: Clarification of low recoveries when measuring concentrations may assist in upgrading this study.

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1010:

- Size of the test organisms is not provided in the Study Report.
- Fortified laboratory well water was used in the study for the dilution water. The guidelines recommend surface or ground water, reconstituted water, deionized water, or dechlorinated tap water.
- Duration of transition from light to dark period not reported.
- It was not reported if the test vessels were covered during the test.
- The guidelines recommend that the concentrations in replicates vary no more than ± 20%. The concentrations in the study were not measured in the replicates, but only in one sample for each treatment level and the control. Further, measured concentrations were, in all levels measure, were below nominal concentrations.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • *Daphnia magna* • *D. D.pulex*	• Water Fleas (*Daphnia magna*) (p. 8)
Life Stage • 1st instar (≤24 h)	• ≤24-hr old (p. 8)
All organisms from same source?	• Yes, Springborn Smithers culture facility. (p. 8)

Signs of disease or injury?	• No signs of disease or injury. (p. 12)
Cultures • Do not contain ephippia	• Cultures did not produce ephippia. (p. 12)
Acclimation Period • Minimum 48-hrs	• At least 48 hours (p. 12)
Feeding • No feeding during study.	• Daphnids were not fed during exposure. (p. 12)
Pretest Mortality • No more than 20% mortality 48 hours prior to testing.	• No mortality of the adult stock was observed during the 48 hours prior to the test initiation. (p. 12)

B. Test System

Guideline Criteria	Reported Information
Source of dilution water •Surface or ground water, reconstituted water, deionized water, or dechlorinated tap water.	• Fortified laboratory well water (p. 12)
Does water support test animals without observable signs of stress?	• Yes, several species of daphnids have survived and reproduced for multiple generations in the fortified well water used for the test. (p. 13)
Photoperiod • 16-hr light and 8-hr dark with 15- to 30-minute transition period.	• 16 hours of light and 8 hours of darkness (p. 12,13) • Duration of transition period not reported.
Test Chambers • Material: Glass or stainless steel. • Size: 250 ml. • Loosely covered.	• Glass battery jars. (p. 15) • 1600 mL. (p. 15) • Coverage information not provided in report.1600-mL glass test vessels (p. 15)
Water Temperature • 20 ± 2°C	• Water temperature was 20°C throughout the experiment. (p. 23)
Dissolved Oxygen • Between 60 and 105% saturation • Do not aerate tests.	• Range: 7.6- 9.0 mg/L. DO concentrations were above 60% throughout the test. (p. 23)
Total Hardness • 180 mg/L as $CaCO_3$ (maximum).	• 170 mg/L as $CaCO_3$ (p. 13)
Flow Rate (Flow-through Test) •At least 5X volume of test chamber. •No more than 10% variation between test chambers.	• Provided approximately six solution volume replacements per day. (p. 15) • Flow-splitting accuracy was within 10% of the targeted delivery. (p. 15)
Solvents •Not to exceed 100 mg/L.	• Acetone: 0.10 mL/L (p. 15)

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test •Widely-spaced concentrations (e.g., 1, 10, 100 mg/L). •Minimum 5 daphnids per concentration.	• Concentrations used in study were based on the results of a chronic flow-through exposure of daphnids to R107894 conducted at Springborn Smithers (Study No. 13751.6145). (p. 14)
Concentrations of Definitive Test •Control & 5 or more treatment levels •A geometric series with 1.5 to 2.0 progression. •2 or more replicates per dose. •Static test: measured at beginning and end (minimum). •Static renewal test: measured at beginning and end of each renewal period. •Flow-through test: measured in each chamber at beginning of test and at 48 hours, and whenever malfunction detected. •Concentrations in replicates vary no more than ± 20%.	• Control, solvent control and 5 treatment levels. (p. 14) • Geometric series with approx. 2 progression. • 2 replicates per dose (p. 14) • Prior to the test, one sample was removed from each treatment level and control solution and analyzed for R107894 and the degradate CL 322,250. (p.17) • During the test, one water sample (alternating between replicates A and B at each interval) from each treatment level and control solutions was collected and analyzed for R107894 and CL 322,250 at test initiation and test termination. (p. 17) • Concentrations were not measured in the replicates.
Number of Test Organisms • Minimum 20/concentration, may be equally divided among containers. • Loading not to exceed 40 daphnids per liter of test solution in static system. • Loading in flow-through system dependent on flow rate.	• Ten daphnids per replicate test aquarium; 2 replicates per treatment (20 daphnids per treatment level and controls). (p. 15, 16) • Loading not reported.
• **Test organisms randomly or impartially assigned to test vessels?**	• Daphnids were impartially added to intermediate test beakers no more than two at a time. (p. 15)
Duration of Test • 48 hours • Each test chamber checked for immobilized daphnids at 24 and 48 hours.	• Test duration: 48 hours (p. 8) • Number of immobilized daphnids recorded at test initiation, 24 and 48 hours of exposure. (p. 16)
Water Parameter Measurements • Temp, DO and pH: measured at beginning and end of test in each chamber.	• Temperature, DO and pH measured once daily in both replicates of each treatment level and controls. (p. 16)

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	• Yes (p.3-4)
Control Mortality • Not more than 10%.	• No immobilization or adverse effects were observed in the controls. (p. 20)
•Percent Recovery of Chemical	• At test termination, measured concentrations of R107894 ranged from 49 to 56% of the nominal concentrations. (p. 19) • At test termination, measured concentrations of CL 322,250 ranged from 37 to 45% of the nominal R107894 concentrations. (p. 19)
•Raw data included?	• Yes

Dose Response

Mortality

Concentration (µg a.i./L)		Number of Organisms	Cumulative Number Immobilized Daphnids	
			Hour of Study	
Nominal	Mean Measured		24	48
Control	N/A	20	0	0
Solvent Control	N/A	20	0	0
0.63	0.32	20	0	0
1.3	0.64	20	0	3
2.5	1.4	20	4	4
5.0	2.7	20	12	18
10	5.2	20	14	20

Statistical Results

Statistical Method: The mean measured concentrations tested and the corresponding immobilization data were

Statistical Results

Statistical Method: The mean measured concentrations tested and the corresponding immobilization data were used to estimate the 24- and 48-hour EC_{50} and 95% confidence intervals. The 24-EC_{50} and corresponding 95% confidence intervals were determined by probit analysis. The 48-EC_{50} and corresponding 95% confidence intervals were determined by moving average angle analysis. It appears that the NOEC was estimated by visual inspection of the immobilization data.

Results Synopsis:

24-Hour Values

EC_{50} = 2.8 µg a.i./L 95% confidence intervals = 2.2-3.9 µg a.i./L

48-Hour Values

EC_{50} = 1.5 µg a.i./L 95% confidence intervals = 1.2-1.9 µg a.i./L

NOEC: 0.32 µg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Not performed.

14. REVIEWER'S COMMENTS:

No additional comments.

DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** ECONEA Technical **PC Code No.:** 119093

2. **TEST MATERIAL:** R10894 **Purity:** 94.6%

3. **CITATION**

Author:	Hoberg, James R.
Title:	R107894—Acute Toxicity to the Marine Diatom, *Skeletonema costatum,* Under Static Conditions
Study Completion Date:	March 17, 2005
Laboratory:	Springborn Smithers Laboratories, 790 Main St. Wareham MA 02571-1075
Sponsor:	Janssen Pharmaceutica NV, Plant and Material Protection Division, Turnhoutseweg 30, B-2340 Beerse, Belgium
Laboratory Report ID:	13751.6147
DP Barcode:	321453
MRID No.:	466199-01

4. **REVIEWED BY:**

Signature: Date: 1/19/06

David C. Bays, RASSB, AD (7510C)

5. **APPROVED BY:**

Signature: Date: 1/19/06

Rick Petrie, Team 3 Leader, RASSB, AD (7510C) HCook for

Kathryn Montague, Acting Team 1 Leader, RASSB, AD (7510C) HCook for

6. **STUDY PARAMETERS**

Definitive Test Duration: 96-hour
Type of Concentrations: Nominal

7. **CONCLUSIONS**

Results Synopsis: A significant reduction in cell density was detected in treatment levels 0.0034 mg a.i./L. Based on the Williams' Test, the 96-hour NOEC was determined to be 0.0015 mg a.i./L. The 96-hour EC50 value was determined to be 0.0027 mg a.i./L, with 95% confidence intervals of 0.0026 to 0.0029 mg a.i./L.

Verified Results Synopsis: No calculation errors were found in the review of statistical calculations. The Dunnet's test showed statistically significant differences in the same dose groups as the study author's Williams' test.

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Scientifically sound study

C. Repairability: Not Applicable

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.5400:

- The temperature fell outside the range of 20±2°C on days 3 and 4, when the solution temperature ranged from 17 to 19°C.
- The light intensity fell outside the range of 4.3 k Lx ± 10% on days 2 and 3, when the light intensity at five of the 24 vessels was measured to be 445 to 468 footcandles (4.8 to 5.0 K lx).
- The following items were not reported in the study report:
  - Sterilization/cleaning practices
  - Water solubility
  - Physical/chemical properties of the chemical, including saturation concentration
  - ~~The maximum labeled rate~~ MCook
- The lowest concentration of the range-finding test (0.0010 mg a.i./L) was not at the detection limit (0.000011 mg a.i./L).
- ~~Only two replicates per dose/control group were used in the range-finding test, instead of three.~~ MCook
- Doses selected for the main test progressed by factors of 2.5-2.6 times, rather than 1.5-2 times.
- ~~No positive control was used.~~ MCook

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information

Species •*Selenastrum capricarnatum (Raphidacelis subcapitata)* •*Skeletanema castatum* •*Anabaena flas-aquae* •*Navicula pelliculasa*	*Skeletanema castatum* was used.
Initial Number of Cells •10,000 cells/mL (*Selenastrum, Anabaena, Navicula)* •77,000 cells/mL (*Skeletanema*)	Approximately 77,000 cells/mL. p15
Stock Culture •3 to 7 days old	Three days. p13
Nutrients •Standard formula (ASTM E1218-20) •pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) •Freshly prepared	• Sterile medium used • pH=8.1± 0.1

B. Test System

Guideline Criteria	Reported Information
Solvent Upper limit - 0.5 mL/L	• 0.1 mL/L. p15
Temperature •24° ± 2°C (*Selenastrum, Navicula, Anabaena*) •20° ± 2°C (*Skeletanema*) •Recorded hourly	• 20°C±2°C, except between days 3 and 4, when the solution temperature ranged from 17 to 19°C. p23,27 • Temperature recorded continuously. p16
Light Intensity •4.3 K lx (±10%) (*Selenastrum, Skeletonema, Navicula*) •2.2 K lx (±10%) (*Anabaena*) •Photosynthetically active radiation approx. 66.5 ± 10% μEin/m^2/sec	• 3.9 to 4.7 K lx, except between the 48- and 72-hr observation period, when the light intensity at five of the 24 vessels was measured to be 445 to 468 footcandles (4.8 to 5.0 K lx). p23, 27
Photoperiod •14-hr light/10-hr dark (*Skeletanema*) •Continuous (*Selenastrum, Navicula, Anabaena*)	14-hr light/10-hr dark used. p16
pH •pH of nutrient medium: 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*) 8.1 ± 0.1 (*Skeletanema*) •Measured at beginning and end of test	• Nutrient medium pH = 8.1±0.1. p13 • Measured at beginning and end of test. p27
Oscillation Rates •100 cycles/min (*Selenastrum*) •60 cycles/min (*Skeletanema*)	• 60±10 rpm. p13

Guideline Criteria	Reported Information
Test Containers •125-500 mL Erlenmeyer flasks •Cleaned/sterilized (solvent and acid) and conditioned •Test solution volume ≤ 50% of flask volume	• 250 mL Erlenmeyer flasks. p15 • Conditioned, but sterilization/cleaning not reported • Test solution volume = 100 mL. p15
Dilution Water •Sufficient quality (e.g., ASTM Type I) •Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	• Artificially enriched seawater used (salinity = 30±2 g/L). p13

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test •Water solubility and physical-chemical properties of test chemical determined? •Validated analytical method developed? •Expose algae to widely spaced (e.g. log interval) chemical concentration series •Lowest value should be at detection limit •Upper value, for water soluble compounds, should be at saturation concentration •Minimum of 3 replicates •Algae should be exposed for 96 hours •If highest concentration (saturation concentration or 100 mg/L) results in <50% reduction in growth, definitive test may not be necessary •If lowest concentration (detection limit) results in >50% reduction, definitive test necessary	• Water solubility, physical/chemical properties could not be found in the study report. p19 • Validated method. p48 • Log intervals used. p19 • Lowest concentration of range-finding test (0.0010 mg a.i./L) (p19) not at detection limit (0.000011 mg a.i./L).p54 Saturation concentration not reported. • Two replicates per dose/control group. p19 • 96 hours of exposure • Definitive test justified based on results from range finding test
Dose Range •1.5X -2X progression	• 2.5X-2.6X progression calculated from doses
Doses •5 or more concentrations of test substance in a geometric series •> 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	• 6 doses in a geometric series • 100% inhibition at highest doses. p30
Controls •Negative and/or solvent each test •Positive - zinc chloride (periodically)	• Negative and solvent controls used • No positive control
Replicates Per Dose •3 or more (4 or more for *Navicula*)	• Three replicates/dose. p15

Duration of Test •96-hr	• 96 hour duration.
Growth •Logarithmic growth (controls) by 96-hr or repeat test (increase by a factor of 16) •1.5 x 10^6 cells/mL (*Skeletonema*) •3.5 x 10^6 cells/mL (*Selenastrum*)	• Increase by more than a factor of 16. 1.55x10^6 cell/mL at 96 hrs. p30
•Daily Observations?	Yes. p16
Method of Observations •Direct - microscopic cell count of at least 400 cells/flask •Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count •Qualitative and descriptive	Direct method used. p15 At least 400 cells counted. p16
Cell Separation •Syringe ultrasonic bath, or blender; limited sonification (*Anabaena*) •Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula)*	No report of filament-breaking could be found in the study report.
•Algistatic and algicidal effects differentiated?	Yes. p16
•Maximum Labeled Rate	It is unclear if the maximum labeled rate was used.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	Yes. p13
Growth in controls reported?	Yes. p30
Description of test system and test design included?	Yes
Initial and final chemical concentrations and pH measured?	Yes
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	Yes
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	Yes
Raw data included?	Yes. p30
Methods and data records reported?	Yes. p18, appendix 2

Statistical Analysis •Mean and standard deviation calculated and plotted? •Goodness-of-fit determined?	Yes.

Dose Response

Nominal Concentration (mg/L)	Initial Measured Concentration (mg/L)	Final Measured Concentration (mg/L)	Cell Density at 96 hrs ($\times 10^4$ cells/mL)	% Inhibition (reduction in growth rate compared with Solvent Control)	pH	
					0-hr	96-hr
Control	<0.00016	<0.00016	162.56±11.67	NA	7.9	8.8
Solvent Control	<0.00016	<0.00016	138.89±4.91	NA	8.0	8.9
0.0010	0.0010	<0.00016	164.67±4.36	-9	8.0	8.9
0.0026	0.0025	<0.00039	133.94±14.17	11	8.0	8.8
0.0064	0.0058	<0.00079	146.89±17.45	3	8.1	9.0
0.016	0.015	<0.0016	41.33±14.86	73	8.1	8.4
0.040	0.037	<0.0039	0.17±0.29	100	8.1	8.0
0.10	0.089	<0.0079	0.00±0.00	100	8.0	8.0

Statistical Results

Statistical Method: A t-test was used to compare the daily cell density of the control to the solvent control. The solvent control was used for comparison to treatment data if a significant difference was determined; otherwise, the control and solvent control data were pooled and used for comparison. EC50 values were calculated using TOXSTAT. The NOEC was determined by determining the highest test concentration which demonstrated no statistically adverse effect (p 0.05). Normality was checked using Shapiro-Wilks' Test, and homogeneity of variance was checked using Bartlett's Test. If the data sets passed the test for homogeneity and normality, then Williams' Test was used to determine the NOEC. (p. 18)

Results Synopsis: Because a significant difference was determined between the control and solvent control data, the solvent control was used for comparison to treatment data. The cell density data were found to be normally distributed and have homogeneity of variance; therefore, the Williams' Test was used to determine treatment-related effects. A significant reduction in cell density was detected in treatment levels 0.0034 mg a.i./L. Based on the Williams' Test, the 96-hour NOEC was determined to be 0.0015 mg a.i./L. The 96-hour EC50 value was determined to be 0.0027 mg a.i./L, with 95% confidence intervals of 0.0026 to 0.0029 mg a.i./L.

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Calculations of cell density averages and standard deviations were checked by Versar for accuracy. EC50 calculations were inspected for reasonableness with respect to the raw data. In order to verify calculations of the 96-hr NOEC, the Dunnet's test ($p<0.05$) was performed on the cell density data.
Results Verification Synopsis: No calculation errors were found in the review of statistical calculations. The Dunnet's test showed statistically significant differences in the same dose groups as the study author's Williams' test.

14. REVIEWER'S COMMENTS:

The following guideline deviations were found in the study report:

- The temperature fell outside the range of 20±2 °C on days 3 and 4, when the solution temperature ranged from 17 to 19 °C.
- The light intensity fell outside the range of 4.3 k Lx ± 10% on days 2 and 3, when the light intensity at five of the 24 vessels was measured to be 445 to 468 footcandles (4.8 to 5.0 K lx).
- The following items were not reported in the study report:
  - Sterilization/cleaning practices
  - Water solubility
  - Physical/chemical properties of the chemical, including saturation concentration
  - ~~The maximum labeled rate.~~
- The lowest concentration of the range-finding test (0.0010 mg a.i./L) was not at the detection limit (0.000011 mg a.i./L).
- ~~Only two replicates per dose/control group were used in the range-finding test, instead of three.~~
- Doses selected for the main test progressed by factors of 2.5-2.6 times, rather than 1.5-2 times.
- ~~No positive control was used.~~

EPA
United States
Environmental Protection
Agency

Date: 4/11/06

SUBJECT: **Econea Ecotoxicity Studies Submitted in Support of Antifoulant Paint Use**

DP Barcode: 327255
PC Code: 119093

FROM: Richard C. Petrie, Team 3 Leader, Agronomist 4/11/06
OPP/AD/RASSB
Antimicrobial Division (7501C)

THRU: Norm Cook. 4/12/06
Chief, RASSB
Antimicrobial Division (7501C)

TO: Marshall Swindell, RM 33
Antimicrobial Division (7501C)

The RASSB has reviewed 4 ecotoxicity studies submitted in support of chlorfenapyr (Econea) registration as an antifoulant paint:

1.) MRID 465960-05, acute dietary LC50 study using the Mallard duck (*Anas platyrhynchos*). Test chemical was R107894, 94.6%. The dietary LC50: 10.8 ppm a.i. This study is Core.

2.) MRID 465960-13, acute dietary LC50 study using the Mallard duck (*Anas platyrhynchos*). Test chemical was CL322,250 degradate, 88.2%. The dietary LC50: 962.0 ppm a.i. This study is Core.

3.) MRID 465960-11, life cycle study using *Daphnia magna*. The test chemical was CL322,250 degradate, 92.6% in a 21 day flow-through system. The NOAEC: 0.30 mg a.i./L, LOAEC: 0.54 mg a.i./L. This study is Supplemental - upgrade to core is possible upon submission of missing data.

4.) MRID 465960-12, life cycle study using mysid shrimp (*Americamysis bahia*). Test chemical was CL322,250 degradate, 88.2%, in a 28 day flow-through system. This study is Invalid.

If you have any questions, please contact Richard Petrie at Petrie.Rick@epa.gov or (703) 305-7358. DERs are attached.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MARCH 30, 2006

MEMORANDUM

Subject: Review of three acute aquatic toxicity studies, using *Oncorhynchus mykiss, Lepomis macrochirus, and Daphnia magna* as test organisms, submitted to support the proposed registration of CL 322,250 a major degradate of Econea Technical. (DP Barcode 327256; Decision# 220066; PC Code 119093)

From: David C. Bays, Risk Assessment and Science Support Branch (RASSB), Antimicrobials Division (7510W)

To: Marshall Swindell, Product Manager #33, Antimicrobials Division (7510W)

Thru: Norm Cook, Branch Chief, RASSB, AD

RASSB has completed the review of three aquatic toxicity studies (MRIDs 46596008, 46596009 and 46596010) with CL 322,250 a major degradate of Econea Technical as the test chemical. Econea Technical is used as an anti-foulant paint product. The first study was an acute aquatic invertebrate acute toxicity test using Freshwater Daphnids, *Daphnia magna*, as the test organism (OPPTS 850.1010). There were some guideline deviations identified by the reviewer, but these were minor in nature and did not affect the results of the study (see DER for MRID 46596008). Therefore, the study is classified as core and can be used in a risk assessment. As reported, the results were as follows: 48-hour EC_{50} was 0.51 mg a.i./L (95% C.I. = 0.42-0.61 mg a.i./L) and the NOEC was 0.25 mg a.i./L, which indicates that CL 322,250 is acutely highly toxic to freshwater daphnids.

The second study (MRID 46596009) was a fish acute toxicity test using Bluegill Sunfish, *Lepomis macrochirus*, as the test organism (OPPTS 850.1075). There were some guideline deviations identified by the reviewer, but these were minor in nature and did not affect the results of the study (see DER for MRID 46596009). Therefore, the study is classified as core and its results can be used in a risk assessment. As reported, the results were as follows: 96-hour LC50 was 1.2 mg a.i./L (95% C.I. = 1.1-1.4 mg a.i./L) and the 96-hour NOEC was 0.55 mg a.i./L, which indicates that CL 322,250 is acutely moderately toxic to bluegill sunfish.

The third study (MRID 46596010) was a fish acute toxicity test using Rainbow Trout, *Oncorhnchus mykiss,* as the test organism (OPPTS 850.1735). There were some guideline deviations identified by the reviewer, but these were minor in nature and did not affect the results of the study (see DER for MRID 46596010). Therefore, the study is classified as core and can be used in a risk assessment. As reported, the results were as follows: 96-hour LC50 was 520 µg a.i./L (95% C.I. = 320-870 µg a.i./L) and the NOEC was 320 µg a.i./L, which indicates that CL 322,250 is acutely highly toxic to rainbow trout.

If you have any questions on the above, please contact David Bays at 703-605-0216.

Decision #: 220066

DATA PACKAGE BEAN SHEET

DP #: (327256)

Date: 24-May-2006

* * * Registration Information * * *

Registration: 43813-ET - ECONEA TECHNICAL

Company: 43813 - JANSSEN PHARMACEUTICA INC.

Risk Manager: RM 33 - Marshall Swindell - (703) 308-6341 Room# PY1 S-8828

Risk Manager Reviewer: Norman Cook NCOOK

Sent Date: 12-May-2005 Calculated Due Date: 08-Jan-2007 Edited Due Date:

Type of Registration: Product Registration - Section 3

Action Desc: (A41) NEW AI;NON-FOOD USE;OUTDOOR;OTHER USES;

Ingredients: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-(93.2%)

* * * Data Package Information * * *

Expedite: Yes ● No Date Sent: 27-Feb-2006 Due Back:

DP Ingredient: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-

DP Title:

CSF Included: Yes ● No Label Included: Yes ● No Parent DP #: 321452

Assigned To	Date In	Date Out	
Organization: AD / RASSB	27-Feb-2006	24-May-2006	Last Possible Science Due Date: 03-Jul-2006
Team Name: RASSB3	27-Feb-2006	24-May-2006	Science Due Date:
Reviewer Name: Bays, David	27-Feb-2006	24-May-2006	Sub Data Package Due Date:
Contractor Name:			

* * * Studies Sent for Review * * *

No Studies

* * * Additional Data Package for this Decision * * *

Printed on Page 2

* * * Data Package Instructions * * *

Sub-bean for review of MRIDs 46596010, 46596011, 46596012, and 46596013. NCook

DP#: (327256) *** Additional Data Package for this Decision *** Decision#: (220066)

DP #	Division/Branch	Date Sent	Date Due	Instructions?	CSF	label
289021	AD / RMB1	20-Mar-2003	03-Jul-2006	Yes No	Yes No	Yes No
289021	AD / RASSB	20-Mar-2003	03-Jul-2006	Yes No	Yes No	Yes No
289026	AD / RMB1	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
289026	AD / RASSB	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
289027	AD / RMB1	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
289027	AD / RASSB	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
289029	AD / RMB1	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
289029	AD / RASSB	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
289031	AD / RMB1	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
289031	AD / RASSB	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
289033	AD / RMB1	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
289033	AD / RASSB	20-Mar-2003	24-Dec-2006	Yes No	Yes No	Yes No
290345	AD / RMB1	28-May-2003	24-Dec-2006	Yes No	Yes No	Yes No
290345	AD / RASSB	28-May-2003	24-Dec-2006	Yes No	Yes No	Yes No
292015	AD / RASSB	16-Jul-2003	24-Dec-2006	Yes No	Yes No	Yes No
292015	AD / RASSB	16-Jul-2003	24-Dec-2006	Yes No	Yes No	Yes No
295928	AD / RASSB	07-Nov-2003	03-Jul-2006	Yes No	Yes No	Yes No
295928	AD / RASSB	07-Nov-2003	03-Jul-2006	Yes No	Yes No	Yes No
301745	AD / RASSB	26-Apr-2004	03-Jul-2006	Yes No	Yes No	Yes No
301745	AD / RASSB	26-Apr-2004	03-Jul-2006	Yes No	Yes No	Yes No
312232	AD / RMB1	14-Jan-2005	03-Jul-2006	Yes No	Yes No	Yes No
312232	AD / RASSB	14-Jan-2005	03-Jul-2006	Yes No	Yes No	Yes No
312744	AD / RMB1	04-Feb-2005	03-Jul-2006	Yes No	Yes No	Yes No
312744	AD / RASSB	04-Feb-2005	03-Jul-2006	Yes No	Yes No	Yes No
315652	AD / RASSB	06-Apr-2005	03-Jul-2006	Yes No	Yes No	Yes No
315652	AD / RASSB	06-Apr-2005	03-Jul-2006	Yes No	Yes No	Yes No
315657	AD / RASSB	06-Apr-2005	03-Jul-2006	Yes No	Yes No	Yes No
315657	AD / RASSB	06-Apr-2005	03-Jul-2006	Yes No	Yes No	Yes No
315655	EFED / EISB	06-Apr-2005	03-Jul-2006	Yes No	Yes No	Yes No
315655	AD / RASSB	06-Apr-2005	03-Jul-2006	Yes No	Yes No	Yes No
315656	EFED / ID	06-Apr-2005	03-Jul-2006	Yes No	Yes No	Yes No
315656	AD / RASSB	06-Apr-2005	03-Jul-2006	Yes No	Yes No	Yes No
316718	AD / RMB1	06-May-2005	03-Jul-2006	Yes No	Yes No	Yes No
316718	AD / RASSB	06-May-2005	03-Jul-2006	Yes No	Yes No	Yes No
318296	AD / RMB1	15-Jun-2005	08-Dec-2006	Yes No	Yes No	Yes No
318296	AD / PSB	15-Jun-2005	08-Dec-2006	Yes No	Yes No	Yes No
321452	AD / RMB1	08-Sep-2005	03-Jul-2006	Yes No	Yes No	Yes No
321452	AD / RASSB	08-Sep-2005	03-Jul-2006	Yes No	Yes No	Yes No
321453	AD / RMB1	08-Sep-2005	03-Jul-2006	Yes No	Yes No	Yes No
321453	AD / RASSB	08-Sep-2005	03-Jul-2006	Yes No	Yes No	Yes No
323129	AD / RMB1	31-Oct-2005	03-Jul-2006	Yes No	Yes No	Yes No
323129	AD / RASSB	31-Oct-2005	03-Jul-2006	Yes No	Yes No	Yes No
323132	AD / RMB1	31-Oct-2005	03-Jul-2006	Yes No	Yes No	Yes No
323132	AD / RASSB	31-Oct-2005	03-Jul-2006	Yes No	Yes No	Yes No
325938	AD / RASSB	30-Jan-2006	03-Jul-2006	Yes No	Yes No	Yes No
325938	AD / RASSB	30-Jan-2006	03-Jul-2006	Yes No	Yes No	Yes No
327255	AD / RASSB	27-Feb-2006	03-Jul-2006	Yes No	Yes No	Yes No
327255	AD / RASSB	27-Feb-2006	03-Jul-2006	Yes No	Yes No	Yes No
327534	AD / RMB1	08-Mar-2006	03-Jul-2006	Yes No	Yes No	Yes No
327534	AD / RASSB	08-Mar-2006	03-Jul-2006	Yes No	Yes No	Yes No
327535	AD / RMB1	08-Mar-2006	03-Jul-2006	Yes No	Yes No	Yes No
327535	AD / RASSB	08-Mar-2006	03-Jul-2006	Yes No	Yes No	Yes No
327536	AD / RMB1	08-Mar-2006	03-Jul-2006	Yes No	Yes No	Yes No
327536	AD / RASSB	08-Mar-2006	03-Jul-2006	Yes No	Yes No	Yes No
327537	AD / RMB1	08-Mar-2006	03-Jul-2006	Yes No	Yes No	Yes No

DP#: (327256) *** Additional Data Package for this Decision *** Decision#: (220066)

DP #	Division/Branch	Date Sent	Date Due	Instructions?	CSF	label
327537	AD / RASSB	08-Mar-2006	03-Jul-2006	○ Yes ○ No	○ Yes ● No	● Yes ○ No
327538	AD / RMB1	08-Mar-2006	03-Jul-2006	○ Yes ○ No	○ Yes ● No	● Yes ○ No
327538	AD / RASSB	08-Mar-2006	03-Jul-2006	○ Yes ○ No	○ Yes ● No	● Yes ○ No
328525	AD / RMB1	18-Apr-2006	18-Aug-2006	○ Yes ○ No	○ Yes ● No	● Yes ○ No
328525	AD / PSB	18-Apr-2006	18-Aug-2006	○ Yes ○ No	○ Yes ● No	● Yes ○ No
328778	AD / RMB1	25-Apr-2006	03-Jul-2006	○ Yes ○ No	○ Yes ● No	○ Yes ● No
328778	AD / RASSB	25-Apr-2006	03-Jul-2006	○ Yes ○ No	○ Yes ● No	○ Yes ● No

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE ACUTE TOXICITY TEST, FRESHWATER DAPHNIDS
GUIDELINE OPPTS 850.1010

1. **CHEMICAL:** ECONEA Technical **PC Code No.:** 119093

2. **TEST MATERIAL:** CL 322,250 **Purity:** 92.6%
Lot or Batch No.: AC12395-43

3. **CITATION**

Authors:	Mark A. Cafarella
Title:	CL 322,250-Acute Toxicity to Water Fleas, (*Daphnia Magna*) Under Flow-Through Conditions
Study Completion Date:	June 28, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6151 Sponsor Protocol/Project No. AGR 925
MRID No.:	465960-08

4. **REVIEWED BY:**

Signature: **Date:** 3/30/06
David Bays, Microbiologist, RASSB, AD

5. **APPROVED BY:**

Signature: **Date:** 3/30/06
Norm Cook, Branch Chief, RASSB, AD

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Daphnia magna*
Age of Test Organism:	<24 hours
Definitive Test Duration:	48 hours (May 17-19, 2005)
Study Method:	Flow-through
Type of Concentrations:	Both (results based on mean-measured concentrations)

7. CONCLUSIONS

Results Synopsis:

48-Hour Values
EC_{50} = 0.51 mg a.i/L
95% confidence intervals = 0.42 to 0.61 mg a.i./L

NOEC= 0.25 mg a.i./L

8. ADEQUACY OF THE STUDY

A. **Classification:** Core

B. **Rationale:** Minor guideline deviations that should not affect the results of the study

C. **Repairability:** N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1010:

- Size of the test organisms is not provided in the Study Report.
- Fortified laboratory well water was used in the study for the dilution water. The guidelines recommend surface or ground water, reconstituted water, deionized water, or dechlorinated tap water.
- The exact transition period was not reported.
- The coverage for the test containers was not provided in the Study Report.
- The guidelines recommend that the concentrations in replicates vary no more than ± 20%. The concentrations in the study were not measured in the replicates, but only in one sample for each treatment level and the control.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ *Daphnia magna* ▪ *D. pulex*	▪ *Daphnia magna* (p. 9)
Life Stage ▪ 1st instar (24 h)	▪ Yes, <24 hours. (p. 9)
All organisms from same source?	▪ Yes, Springborn Smithers culture facility. (p. 9)
Organisms approximately same size and age?	▪ Organisms were <24 hours. Size of the test organisms is not provided in the study report. (p. 12)
Signs of disease or injury?	▪ No signs of disease or injury. (p. 12)
Cultures ▪ Do not contain ephippia	▪ No ephippia was produced. (p. 12)
Acclimation Period ▪ Minimum 48-hrs	▪ Yes, 48 hours. (p. 12)
Feeding ▪ No feeding during study.	▪ Daphnids were not fed during the exposure. (p. 13)
Pretest Mortality ▪ No more than 20% mortality 48 hours prior to testing.	▪ No mortality was observed during the 48 hours prior to test initiation. (p. 12)

B. Test System

Guideline Criteria	Reported Information
Source of dilution water ▪ Surface or ground water, reconstituted water, deionized water, or dechlorinated tap water.	▪ Fortified laboratory well water. (p. 13)
Does water support test animals without observable signs of stress?	▪ Yes. (p. 13-14, 25)
Photoperiod ▪ 16-hr light and 8-hr dark with 15- to 30-minute transition period.	▪ 16-hr light and 8-hr dark and sudden transitions from light to dark and vice versa were avoided. (p. 13-14)
Test Chambers ▪ Material: Glass or stainless steel. ▪ Size: 250 ml. ▪ Loosely covered.	▪ Glass battery jars. (p. 15) ▪ 1600 mL. (p. 15) ▪ Coverage information not provided in report.
Water Temperature ▪ 20 ± 2°C	▪ 20 ± 2°C (p. 14, 18)

Dissolved Oxygen • Between 60 and 105% saturation • Do not aerate tests.	• 8.6 to 9.0 mg/L. Greater than 60% saturation. (p. 23) • Aeration was not discussed in the report.
Total Hardness • 180 mg/L as $CaCO_3$ (maximum).	• Ranged from 170 to 180 mg/L as $CaCO_3$ (p. 13)
Flow Rate (Flow-through Test) • At least 5X volume of test chamber. • No more than 10% variation between test chambers.	• Provided approximately six solution volume replacements per day. (p. 15) • Flow-splitting accuracy was within 10% of the targeted delivery. (p. 15)
Solvents • Not to exceed 100 mg/L.	• Use of solvents was not reported.

C. **Test Design**

Guideline Criteria	Reported Information
Range-Finding Test • Widely-spaced concentrations (e.g., 1, 10, 100 mg/L). • Minimum 5 daphnids per concentration.	• Concentrations used in study were based on the results of a chronic flow-through exposure of daphnids to CL322, 250 conducted at Springborn Smithers (Study No. 13751.6152). (p. 14) • The protocol found in the report follows the guideline (p. 31)
Concentrations of Definitive Test • Control & 5 or more treatment levels • A geometric series with 1.5 to 2.0 progression. • 2 or more replicates per dose. • Static test: measured at beginning and end (minimum). • Static renewal test: measured at beginning and end of each renewal period. • Flow-through test: measured in each chamber at beginning of test and at 48 hours, and whenever malfunction detected. • Concentrations in replicates vary no more than ± 20%.	• Yes (control, 0.31, 0.63, 1.3, 2.5, and 5.0 mg a.i./L). (p. 14) • Yes. (p. 14) • 2 replicates for each treatment level and the control. (p. 14) • One samples from each treatment level, the control, and three quality control samples measured at 0 and 48 hours. (p. 16-17, 24) • Concentrations were not measured in the replicates.
Number of Test Organisms • Minimum 20/concentration, may be equally divided among containers • Loading not to exceed 40 daphnids per liter of test solution in static system • Loading in flow-through system dependent on flow rate.	• Yes. (10 daphnids per vessel and two replicates per treatment level). (p. 16) • Daphnids were added no more than two at a time. Flow provided a 90% test solution replacement rate of approximately 9 hours. (p. 15)
Test organisms randomly or impartially assigned to test vessels?	• Yes. (p. 16)

Duration of Test • 48 hours • Each test chamber checked for immobilized daphnids at 24 and 48 hours.	• 48 hours. (p. 16) • Yes. (p. 16)
Water Parameter Measurements • Temp, DO and pH: measured at beginning and end of test in each chamber.	• Yes. Measured at 0, 24, and 48 hours in each chamber. (p. 16, 23)

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	• Yes. (p. 3, 4)
Control Mortality • Not more than 10%.	• No immobilization or adverse effects were observed in the control groups. (p. 19, 25)
Percent Recovery of Chemical	• Percent of nominal ranged from 79 to 110%. Percent recovery based on quality control samples ranged from 95.1 to 101%. (p. 19, 24)
Raw data included?	• Yes. (p. 23-25,)

Dose Response

Mortality:

Concentration (ppm)		Number of Organisms	Cumulative Number Dead	
Nominal (mg a.i./L)	Mean Measured (mg a.i./L)		Hour of Study	
			24	48
Control	Control	20	0	0
0.31	0.25	20	0	0
0.63	0.53	20	0	11
1.3	1.4	20	20	20
2.5	2.7	20	20	20
5.0	5.0	20	20	20

Statistical Results

Statistical Method:

The study reported that the mean measured concentrations tested and the corresponding immobilization data were used to estimate the 24- and 48-hour EC_{50} values and 95% confidence intervals. A computer program using binomial probability calculated the EC_{50} values and 95% confidence intervals.

It appears that the NOEC was determined by empirical analysis of the mortality data.

Results Synopsis:

24-Hour Values
EC_{50} = 0.86 mg a.i/L
95% confidence intervals = 0.53 to 1.4 mg a.i./L

48-Hour Values
EC_{50} = 0.50 mg a.i/L
95% confidence intervals = 0.25 to 0.53 mg a.i./L

NOEC= 0.25 mg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Versar calculated the 24- and 48-hour EC_{50} values for the mortality data using linear interpolation and the mean-measured concentrations.

The statistical computer program that determines the NOEC could not be performed because there were only two replicates in the study. The mortality data was empirically analyzed to determine the NOEC.

Results Verification Synopsis:

24-Hour Values
EC_{50} = 0.97 mg a.i/L
95% confidence intervals = 0.97 to 0.97 mg a.i./L

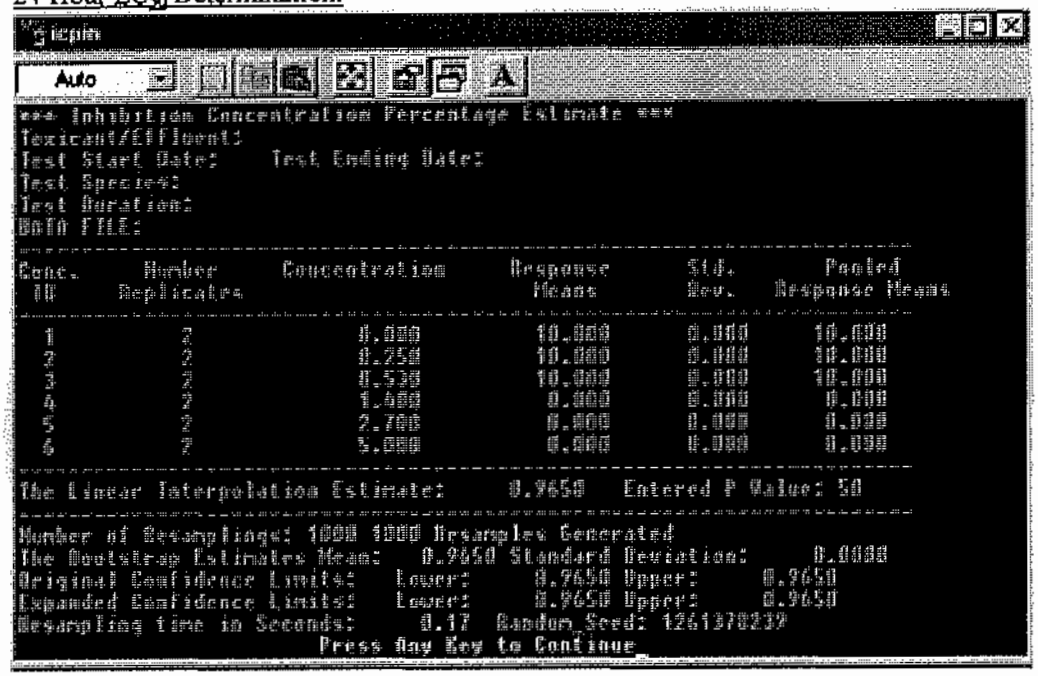
48-Hour Values
EC_{50} = 0.51 mg a.i/L
95% confidence intervals = 0.42 to 0.61 mg a.i./L

NOEC= 0.25 mg a.i./L

14. REVIEWER'S COMMENTS:

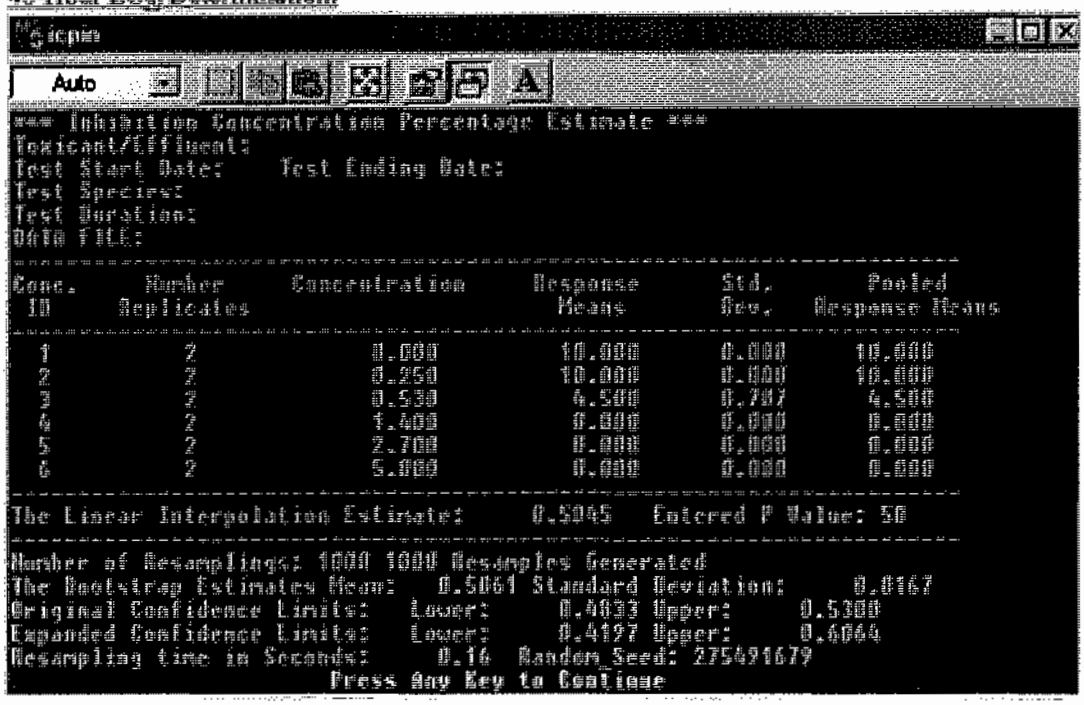
- Guideline deviations are shown in Section 9.
- The 24- and 48-hour EC_{50} values and 95% confidence intervals calculated by Versar were different than those reported by the study author. The differences may be due to the use of different statistical tests.

24-Hour EC_{50} Determination:



```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent:
Test Start Date:     Test Ending Date:
Test Species:
Test Duration:
Data FILE:

Conc.    Number      Concentration      Response      Std.       Pooled
 ID     Replicates                       Means        Dev.    Response Means

  1         2             0.000          10.000       0.000      10.000
  2         2             0.250          10.000       0.000      10.000
  3         2             0.530          10.000       0.000      10.000
  4         2             1.400           0.000       0.000       0.000
  5         2             2.700           0.000       0.000       0.000
  6         2             5.000           0.000       0.000       0.000

The Linear Interpolation Estimate:     0.9650    Entered P Value: 50

Number of Resamplings: 1000 1000 Resamples Generated
The Bootstrap Estimates Mean:    0.9650 Standard Deviation:      0.0000
Original Confidence Limits:   Lower:      0.9650 Upper:      0.9650
Expanded Confidence Limits:   Lower:      0.9650 Upper:      0.9650
Resampling time in Seconds:      0.17  Random Seed: 1261370239
                    Press Any Key to Continue
```

48-Hour EC_{50} Determination:



```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent:
Test Start Date:     Test Ending Date:
Test Species:
Test Duration:
Data FILE:

Conc.    Number      Concentration      Response      Std.       Pooled
 ID     Replicates                       Means        Dev.    Response Means

  1         2             0.000          10.000       0.000      10.000
  2         2             0.250          10.000       0.000      10.000
  3         2             0.530           4.500       0.707       4.500
  4         2             1.400           0.000       0.000       0.000
  5         2             2.700           0.000       0.000       0.000
  6         2             5.000           0.000       0.000       0.000

The Linear Interpolation Estimate:     0.5045    Entered P Value: 50

Number of Resamplings: 1000 1000 Resamples Generated
The Bootstrap Estimates Mean:    0.5061 Standard Deviation:      0.0167
Original Confidence Limits:   Lower:      0.4833 Upper:      0.5300
Expanded Confidence Limits:   Lower:      0.4127 Upper:      0.6064
Resampling time in Seconds:      0.16  Random Seed: 275491679
                    Press Any Key to Continue
```

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)- (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** CL322,250 **Purity:** 92.6%

3. **CITATION:**

Author:	Arthur E. Pitt
Title:	CL322,250 – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus)* Under Flow-though Conditions
Study Completion Date:	May 9, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1075
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6149 Janessen Study No. AGR 923
MRID No.:	MRID 465960-09

4. **REVIEWED BY:**

   Signature: **Date:** 3/30/06

   David Bays, Microbiologist, RASSB, AD

5. **APPROVED BY:**

   Signature: **Date:** 3/30/06

   Norm Cook, Branch Chief, RASSB, AD

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Lepomis macrochirus*
Age of Test Organism:	Not provided
Definitive Test Duration:	4 days, March 17-21, 2005
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean measured

7. **CONCLUSIONS**

   Results Synopsis:

96-hour LC_{50}: 1.2 mg a.i./L Confidence (95%) interval: 1.1-1.4 mg a.i./L
96-hour NOEC: 0.55 mg a.i./L

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Minor guideline deviations that should not affect the results of the study

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm with a fill volume of 6.8 L. Guidelines state that the aquaria should be 30 x 60 x 20 cm and have a fill volume of 15 to 30 L of solution.
- The biomass loading was 0.35 g/L/day instead of the guideline stipulation of 1 g/L/day.
- The dissolved oxygen level dropped below the 75% guideline stipulation in two replicate chambers of the treatments.
- No statement was made as to the signs of disease 48-hours prior to testing.
- Fish were not noted as either being or not being from the same class year.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • Preferred species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*)	• Bluegill sunfish (*Lepomis macrochirus*)
Mean Weight • 0.5-5 g	• Mean: 1.8 g (p. 9) • Range: 0.90-3.1 g (p. 9)
Mean Standard Length • Longest not > 2x shortest	• Yes, Mean= 49 mm and ranged form 42-60 mm (p. 9)

Supplier	Osage Catfisheries Osage Beach, Missouri (p. 12)
All fish from same source?	Yes (p. 12)
All fish from the same year class?	Not provided

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period • Minimum 14 days	• Yes (p.13)
Wild caught organisms were quarantined for 7 days?	• Not applicable
Were there signs of disease or injury?	• Information not provided (p.13)
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	• No sign of mortality 48-hours prior to testing, no other observations provided. (p. 13)
Feeding • No feeding during the study	• Feeding was not conducted 48 hours prior to testing or during testing. (p. 13)
Pretest Mortality • No more than 3% mortality 48 hours prior to testing	• The mortality rate was 0% 48 hours prior to test initiation. (p.13)

C. Test System

Guideline Criteria	Reported Information
Source of dilution water • Soft reconstituted water or water from a natural source, not dechlorinated tap water	• Yes, well water was utilized. (p. 13)
Does water support test animals without observable signs of stress?	• Yes, freshwater organisms have survived and reproduced for generations in the well water. (p. 13)
Water Temperature • 12°C for cold water species • 17°C or 22°C for warm water species	• Test temperatures were from 22 to 23°C (p. 19)
pH • Prefer 7.2 to 7.6	• pH ranged from 7.3 to 7.8 (p. 23)
Dissolved Oxygen • Flow-through: >75%	• In replicate A of the 0.58 mg/L treatment, the dissolved oxygen concentration dropped to 72% but raised to 77% by scraping microbial growth from aquarium. In replicate B of the 0.97 mg/L treatment, the dissolved oxygen concentration was found to be 73% at test termination. All other replicates were above 75% saturation. (p.19)

Guideline Criteria	Reported Information
Total Hardness • Prefer 40 to 180 mg/L as $CaCO_3$	• Total hardness as calcium carbonate: 52 mg/L. (p.13)
Test Aquaria • Material: Glass or stainless steel • Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm • Fill volume: 15-30 L of solution	• Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm (p. 14 and 15) • Fill Volume: 6.8 L (p.15)
Type of Dilution System • Must provide reproducible supply of toxicant	• Yes, the dilution system was in operation for seven days prior to testing to ensure constant test substance placement. (p.15)
Flow Rate • Consistent flow rate of 5-10 vol/24 hours • Meter systems calibrated before study and checked twice daily during test period	• Constant flow rate at 7.7 volume replacements/ day. • The system was calibrated seven days prior to test initiation and visually inspected twice a day. (p.15)
Biomass Loading Rate • Static: 0.8 g/L at 17°C, 0.5 g/L at > 17°C • Flow-through: 1 g/L/day	• Biomass loading 0.35 g/L/day (p.16)
Photoperiod • 16 hours light, 8 hours dark	• 16 hours light, 8 hours dark (p. 14 and 12)
Solvents • Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	• Acetone: 0.10 mL/L (p. 14 and 15)

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test • If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	• Preliminary test conducted • The nominal concentrations were 0.58, 0.97, 1.6, 2.7, and 4.5 mg a.i./L. • Five test organisms per treatment level. • After 96 hours, 100% mortality in 1.6, 2.7, and 4.5 mg/L treatment levels. No mortality in the 0.58 and 0.97 mg/L treatments. (p. 18 and 19)
Nominal Concentrations of Definitive Test • Control & 5 treatment levels • Dosage should be 60% of the next highest concentration • Concentrations should be in a geometric series	• Control, solvent control, and at 0.35, 0.58, 0.97, 1.6, and 2.7 mg a.i./L. • Nominal concentrations were approximately 60% of the next highest. (p. 15) • Concentrations were in a geometric series.
Number of Test Organisms • Minimum 10/level • May be divided among containers	• 20/level, two test aquaria per treatment level. (p. 16)

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	• Selected impartially (p. 16)
Biological observations made every 24 hours?	Yes, at initiation, 24, 48, 72, and 96 hours. (p. 16)
Water Parameter Measurements • Temperature: Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1 C • DO and pH: Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	• Temperature, DO, and pH measurements were conducted for all treatment levels and aquaria daily. • Test solution temperature continuously measured during test in replicate A of control (p. 16) • Temperatures did not vary more than a degree. (p. 19)
Chemical Analysis • Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	• Prior to initiation, samples taken from replicates of high, medium low and control treatment levels and analyzed (p.17) • Sample of stock solution analyzed during pre-test period (p. 17) • During study, one water sample from 1 replicate of each treatment level and controls collected and analyzed at 0-hr and 96-hr (p. 17) • Samples removed from alte mate replicates and initiation and termination (p. 17)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Percent Recovery of Chemical from Chemical Analysis	Yes, the recovery was between 92-97% (p. 24)
Control Mortality • Not more than 10% control organisms may die or show abnormal behavior.	• No mortality was seen in control or solvent control. (p. 25)
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes, organisms were noted to be dead, lethargic, or dark in coloration. (p. 25)

Dose Response

Mortality

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Number of Fish at Test Initiation (Rep A / Rep B)	Number of Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Control	Control	10/10	0/0	0/0	0/0	0/0
Solvent Control	Solvent Control	10/10	0/0	0/0	0/0	0/0
0.35	0.32	10/10	0/0	0/0	0/0	0/0
0.58	0.55	10/10	0/0	0/0	0/0	0/0
0.97	0.92	10/10	0/0	0/0	1/0	1/0
1.6	1.6	10/10	0/0	6/7	7/7	9/10
2.7	2.5	10/10	10/10	10/10	10/10	10/10

Symptoms

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Symptoms			
		24 hour	48 hour	72 hour	96 hour
Control	Control	0	0	0	0
Solvent Control	Solvent Control	0	0	0	0
0.35	0.32	0	0	0	0
0.58	0.55	0	0	0	0
0.97	0.92	0	0	0	0
1.6	1.6	0	2[a]	2[a,b]	1[a]
2.7	2.5	0	0	0	0

a Observed to be lethargic
b Observed to be dark in coloration

Statistical Results

Statistical Method: The 24- and 48-hour LC_{50}'s were estimated using binomial probability. The 72- and 96-hour LC_{50}'s were estimated using probit analysis. The NOEC was estimated by visual inspection. (p. 20)

Results Synopsis:

24-hour LC_{50}:	2.0 mg a.i./L	Confidence (95%) interval:	1.6-2.5 mg a.i./L
48-hour LC_{50}:	1.5 mg a.i./L	Confidence (95%) interval:	0.92-2.5 mg a.i./L
72-hour LC_{50}:	1.4 mg a.i./L	Confidence (95%) interval:	1.2-1.6 mg a.i./L
96-hour LC_{50}:	1.2 mg a.i./L	Confidence (95%) interval:	1.1-1.4 mg a.i./L
96-hour NOEC:	0.55 mg a.i./L		

13. **VERIFICATION OF STATISTICAL RESULTS**
Versar did not verify results.

14. **REVIEWER'S COMMENTS:**
No additional comments.

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)- (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** CL322,250 **Purity:** 92.6%

3. **CITATION:**

Author:	Arthur E. Putt
Title:	CL322,250 – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-though Conditions
Study Completion Date:	April 26, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1075
Sponsor:	Janssen Pharmaceutical N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6150 Janessen Study No. AGR 924
MRID No.:	MRID 465960-10

4. **REVIEWED BY:**

   Signature: **Date:** 3/30/06

   David Bays, Microbiologist, RASSB, AD

5. **APPROVED BY:**

   Signature: **Date:** 3/30/06

   Norm Cook, Branch Chief, RASSB, AD

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Oncorhynchus mykiss*
Age of Test Organism:	Not provided; used juveniles
Definitive Test Duration:	4 days, March 4-8, 2005
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean measured

7. CONCLUSIONS

Results Synopsis:

96-hour LC_{50}: 520 µg a.i./L (Confidence (95%) interval: 320-870 µg a.i./L)
96-hour NOEC: 320 µg a.i./L

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Minor guideline deviations that should not affect the results of the study

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm with a fill volume of 6.8 L were used. Guidelines state that the aquaria should be 30 x 60 x 20 cm and have a fill volume of 15 to 30 L of solution.
- The biomass loading was 0.15 g/L/day, instead of the guideline stipulation of 1 g/L/day.
- No statement was made as to the signs of disease 48-hours prior to testing.
- Fish were not noted as either being or not being from the same class year.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ Preferred species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*)	▪ Rainbow trout (*Oncorhynchus mykiss*)
Mean Weight ▪ 0.5-5 g	▪ Mean: 0.79 g (p. 9) ▪ Range: 0.46-1.17 g (p. 9)

Mean Standard Length • Longest not > 2x shortest	• Yes, Mean= 44 mm and ranged form 36-49 mm (p. 9)
Supplier	Troutlodge, Inc. Sumner, Washington (p. 12)
All fish from same source?	Yes (p. 12)
All fish from the same year class?	Not provided

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period • Minimum 14 days	• Yes (p.13)
Wild caught organisms were quarantined for 7 days?	• Not applicable
Were there signs of disease or injury?	• Information not provided (p.13)
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	• No sign of mortality 48-hours prior to testing, no other observations provided. (p. 13)
Feeding • No feeding during the study	• Feeding was not conducted 48 hours prior to testing or during testing. (p. 13)
Pretest Mortality • No more than 3% mortality 48 hours prior to testing	• The mortality rate was 0% 48 hours prior to test initiation. (p.13)

C. Test System

Guideline Criteria	Reported Information
Source of dilution water • Soft reconstituted water or water from a natural source, not dechlorinated tap water	• Yes, well water was utilized. (p. 13)
Does water support test animals without observable signs of stress?	• Yes, freshwater organisms have survived and reproduced for generations in the well water. (p. 13)
Water Temperature • 12°C for cold water species • 17°C or 22°C for warm water species	• Test temperatures were from 12 to 13°C (p. 24)
pH • Prefer 7.2 to 7.6	• pH ranged from 7.5 to 7.7 (p. 24)
Dissolved Oxygen • Flow-through: >75%	• DO concentrations were above 75% throughout the test. (p.24)

Guideline Criteria	Reported Information
Total Hardness • Prefer 40 to 180 mg/L as $CaCO_3$	• Total hardness as calcium carbonate: 56 mg/L. (p.13)
Test Aquaria • Material: Glass or stainless steel • Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm • Fill volume: 15-30 L of solution	• Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm (p. 14 and 15) • Fill Volume: 6.8 L (p.15)
Type of Dilution System • Must provide reproducible supply of toxicant	• Yes (p.26)
Flow Rate • Consistent flow rate of 5-10 vol/24 hours • Meter systems calibrated before study and checked twice daily during test period	• Constant flow rate at 7.9 vol. replacements/day (p. 15). • The dilution system was calibrated prior to initiation and visually inspected twice a day. (p.15)
Biomass Loading Rate • Static: 0.8 g/L at 17°C, 0.5 g/L at > 17°C • Flow-through: 1 g/L/day	• Biomass loading 0.15 g/L/day (p.16)
Photoperiod • 16 hours light, 8 hours dark	• 16 hours light, 8 hours dark (p. 14 and 12)
Solvents • Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	• Acetone: 0.10 mL/L (p. 14 and 15)

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test • If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	• Preliminary test conducted • The nominal concentrations were 0.52, 0.86, 1.4, 2.4, and 4.0 mg a.i./L. • Five test organisms per treatment level. • After 96 hours, 100% mortality in 0.86, 1.4, 2.4, and 4.0 lm/L treatment levels. Mortality rate of 80% was noted at the lowest treatment level, 0.52 mg/L. (p. 18 and 19) • 2nd test conducted with nominal concentrations of 110, 310, and 810 ug/L and 5 test organisms per treatment level • after 2 hours of exposure, 100% mortality in 860 ug/L treatment level; no mortality in other treatment levels.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test • Control & 5 treatment levels • Dosage should be 60% of the next highest concentration • Concentrations should be in a geometric series	• Control, solvent control, and at 860, 520, 310, 190, and 110 µg a.i./L • Nominal concentrations were approximately 60% of the next highest. (p. 15) • Concentrations were in a geometric series.
Number of Test Organisms • Minimum 10/level • May be divided among containers	• 20/level, two test aquaria per treatment level. (p. 16)
Test organisms randomly or impartially assigned to test vessels?	• Selected impartially (p. 16)
Biological observations made every 24 hours?	Yes, at initiation, 24, 48, 72, and 96 hours. Observed for signs of mortality, with dead fish being removed, and adverse effects. (p. 16)
Water Parameter Measurements • Temperature: Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1 C • DO and pH: Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	• Temperature, DO, and pH measurements were conducted for all treatment levels and aquaria daily. • Test solution temperature continuously measured during test in replicate A of control (p. 16) • Temperatures did not vary more than a degree. (p. 19)
Chemical Analysis • Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	• Prior to initiation, samples taken from replicates of high, medium low and control treatment levels and analyzed (p.17) • Sample of stock solution analyzed during pre-test period (p. 17) • During study, one water sample from 1 replicate of each treatment level and controls collected and analyzed at 0-hr and 96-hr (p. 17) • Samples removed from alternate replicates and initiation and termination (p. 17)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Percent Recovery of Chemical from Chemical Analysis	Yes, 100% of the chemical at all treatment levels was recovered at test termination.

Guideline Criteria	Reported Information
Control Mortality • Not more than 10% control organisms may die or show abnormal behavior.	• No mortality was seen in control or solvent control. (p. 25)
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes, organisms were noted to be dead, lethargic, or dark in coloration. (p. 26)

Dose Response

Mortality

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Number of Fish at Test Initiation (Rep A/ Rep B)	Number of Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Control	Control	10/10	0/0	0/0	0/0	0/0
Solvent Control	Solvent Control	10/10	0/0	0/0	0/0	0/0
110	110	10/10	0/0	0/0	0/0	0/0
190	190	10/10	0/0	0/0	0/0	0/0
310	320	10/10	0/0	0/0	0/0	0/0
520	540	10/10	0/3	2/4	5/6	5/6
860	870	10/10	10/10	10/10	10/10	10/10

Symptoms

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Symptoms			
		24 hour	48 hour	72 hour	96 hour
Control	Control	0	0	0	0
Solvent Control	Solvent Control	0	0	0	0
110	110	0	0	0	0
190	190	0	0	0	0
310	320	0	0	0	0
520	540	1[c] & 1[b]	1[c]	1[c]	1[c] & 2[a]
860	870	0	0	0	O

a Observed to be lethargic
b Observed to be dark in coloration
c Observed to lethargic and dark in coloration

Statistical Results

Statistical Method: All LC_{50} values (24, 48, 72, and 96-hr) and confidence intervals were measured by binominal probability. (p. 27)

Results Synopsis:

24-hour LC_{50}:	640 µg a.i./L	Confidence (95%) interval:	540-870 µg a.i./L
48-hour LC_{50}:	600 µg a.i./L	Confidence (95%) interval:	320-870 µg a.i./L
72-hour LC_{50}:	520 µg a.i./L	Confidence (95%) interval:	320-870 µg a.i./L
96-hour LC_{50}:	520 µg a.i./L	Confidence (95%) interval:	320-870 µg a.i./L
96-hour NOEC:	320 µg a.i./L		

13. VERIFICATION OF STATISTICAL RESULTS

Versar did not verify results.

14. REVIEWER'S COMMENTS:

No additional comments.

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE ACUTE TOXICITY TEST, FRESHWATER DAPHNIDS
GUIDELINE OPPTS 850.1010

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl) (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** R107894 **Purity:** 94.6%
   Lot or Batch No.: AC12649-8

3. **CITATION**

Authors:	Mark A. Cafarella
Title:	R107894 – Acute Toxicity to Water Fleas (*Daphnia magna*) Under Flow-Through Conditions
Study Completion Date:	June 28, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N. V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6141
MRID No.:	465960-01

4. **REVIEWED BY:**

   Signature: **Date:** 10/12/06
   David Bays, Microbiologist, RASSB, AD (7510C)

5. **APPROVED BY:**

   Signature: **Date:** 10/12/06
   Rick Perrie, Team 3 Leader, RASSB, AD (7510C)
   Kay Montague, Acting Team 2 Leader, RASSB, AD (7510C)

6. **STUDY PARAMETERS**

   Scientific Name of Test Organism: *Daphnia magna*
   Age of Test Organism: ≤ 24 hours
   Definitive Test Duration: 48 hours
   Study Method: Flow-through
   Type of Concentrations: Nominal and mean-measured

7. **CONCLUSIONS**

   Results Synopsis:

48-hour EC_{50}:	1.5 µg a.i./L	95% C.I.:	1.2-1.9 µg a.i./L
NOEC:	0.32 µg a.i./L		

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Is a scientifically sound study, but had some guideline deviations that may have affected the results of the study.

C. Repairability: These guideline deviations were corrected by the registrant (See MRID 469179-01 and corrections below)

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1010:

- Size of the test organisms is not provided in the Study Report. **(daphnids were >24 hours old and the same size)**
- Fortified laboratory well water was used in the study for the dilution water. The guidelines recommend surface or ground water, reconstituted water, deionized water, or dechlorinated tap water. **(The water used was filtered well water that is the equivalent of reconstituted water)**
- Duration of transition from light to dark period not reported. **(timers allowed lights to come on/off at staggered times 15 to 30 minutes apart)**
- It was not reported if the test vessels were covered during the test. **(flow-through exposure vessels are seldom covered because any loss of test substance to evaporation is not an issue)**
- The guidelines recommend that the concentrations in replicates vary no more than ± 20%. The concentrations in the study were not measured in the replicates, but only in one sample for each treatment level and the control. Further, measured concentrations were, in all levels measure, were below nominal concentrations. **(Summing the measured concentrations of the parent molecule and its primary degradate yielded total concentrations of >80% of nominal)**

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • *Daphnia magna* • *D. D.pidex*	• Water Fleas (*Daphnia magna*) (p. 8)

Life Stage • 1st instar (#24 h)	• #24-hr old (p. 8)
All organisms from same source?	• Yes, Springborn Smithers culture facility. (p. 8)
Organisms approximately same size and age?	• Daphnids were <24 hours old and the same size.
Signs of disease or injury?	• No signs of disease or injury. (p. 12)
Cultures • Do not contain ephippia	• Cultures did not produce ephippia. (p. 12)
Acclimation Period • Minimum 48-hrs	• At least 48 hours (p. 12)
Feeding • No feeding during study.	• Daphnids were not fed during exposure. (p. 12)
Pretest Mortality • No more than 20% mortality 48 hours prior to testing.	• No mortality of the adult stock was observed during the 48 hours prior to the test initiation. (p. 12)

B. Test System

Guideline Criteria	Reported Information
Source of dilution water XSurface or ground water, reconstituted water, deionized water, or dechlorinated tap water.	• The water was filtered well water, the equivalent of reconstituted water.
Does water support test animals without observable signs of stress?	• Yes, several species of daphnids have survived and reproduced for multiple generations in the fortified well water used for the test. (p. 13)
Photoperiod • 16-hr light and 8-hr dark with 15- to 30-minute transition period.	• 16 hours of light and 8 hours of darkness (p. 12,13) • Timers allowed lights to come on/off a staggered times 15 to 30 minutes apart
Test Chambers • Material: Glass or stainless steel. • Size: 250 ml. • Loosely covered.	• Glass battery jars. (p. 15) • 1600 mL. (p. 15) • Flow-through exposure vessels are seldom covered because any loss of test substance to evaporation is not an issue
Water Temperature • 20 ± 2EC	• Water temperature was 20EC throughout the experiment. (p. 23)
Dissolved Oxygen • Between 60 and 105% saturation • Do not aerate tests.	• Range: 7.6- 9.0 mg/L. DO concentrations were above 60% throughout the test. (p. 23)
Total Hardness • 180 mg/L as $CaCO_3$ (maximum).	• 170 mg/L as $CaCO_3$ (p. 13)

Flow Rate (Flow-through Test) XAt least 5X volume of test chamber. XNo more than 10% variation between test chambers.	• Provided approximately six solution volume replacements per day. (p. 15) • Flow-splitting accuracy was within 10% of the targeted delivery. (p. 15)
Solvents XNot to exceed 100 mg/L.	• Acetone: 0.10 mL/L (p. 15)

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test XWidely-spaced concentrations (e.g., 1, 10, 100 mg/L). XMinimum 5 daphnids per concentration.	• Concentrations used in study were based on the results of a chronic flow-through exposure of daphnids to R107894 conducted at Springborn Smithers (Study No. 13751.6145). (p. 14)
Concentrations of Definitive Test XControl & 5 or more treatment levels XA geometric series with 1.5 to 2.0 progression. X2 or more replicates per dose. XStatic test: measured at beginning and end (minimum). XStatic renewal test: measured at beginning and end of each renewal period. XFlow-through test: measured in each chamber at beginning of test and at 48 hours, and whenever malfunction detected. XConcentrations in replicates vary no more than ± 20%.	• Control, solvent control and 5 treatment levels. (p. 14) • Geometric series with approx. 2 progression. • 2 replicates per dose (p. 14) • Prior to the test, one sample was removed from each treatment level and control solution and analyzed for R107894 and the degradate CL 322,250. (p.17) • During the test, one water sample (alternating between replicates A and B at each interval) from each treatment level and control solutions was collected and analyzed for R107894 and CL 322,250 at test initiation and test termination. (p. 17) • Summing the measured concentrations of the parent molecule and its primary degradate yielded total concentrations of >80% of nominal
Number of Test Organisms • Minimum 20/concentration, may be equally divided among containers. • Loading not to exceed 40 daphnids per liter of test solution in static system. • Loading in flow-through system dependent on flow rate.	• Ten daphnids per replicate test aquarium; 2 replicates per treatment (20 daphnids per treatment level and controls). (p. 15, 16) • Loading not reported.
• **Test organisms randomly or impartially assigned to test vessels?**	• Daphnids were impartially added to intermediate test beakers no more than two at a time. (p. 15)

DP Barcode: MRID No:

Duration of Test • 48 hours • Each test chamber checked for immobilized daphnids at 24 and 48 hours.	• Test duration: 48 hours (p. 8) • Number of immobilized daphnids recorded at test initiation, 24 and 48 hours of exposure. (p. 16)
Water Parameter Measurements • Temp, DO and pH: measured at beginning and end of test in each chamber.	• Temperature, DO and pH measured once daily in both replicates of each treatment level and controls. (p. 16)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	• Yes (p.3-4)
Control Mortality X Not more than 10%.	• No immobilization or adverse effects were observed in the controls. (p. 20)
X**Percent Recovery of Chemical**	• At test termination, measured concentrations of R107894 ranged from 49 to 56% of the nominal concentrations. (p. 19) • At test termination, measured concentrations of CL 322,250 ranged from 37 to 45% of the nominal R107894 concentrations. (p. 19)
X**Raw data included?**	• Yes

Dose Response

Mortality

Concentration (µg a.i./L)		Number of Organisms	Cumulative Number Immobilized Daphnids
Nominal	Mean Measured		Hour of Study

			24	48
Control	N/A	20	0	0
Solvent Control	N/A	20	0	0
0.63	0.32	20	0	0
1.3	0.64	20	0	3
2.5	1.4	20	4	4
5.0	2.7	20	12	18
10	5.2	20	14	20

Statistical Results

Statistical Method: The mean measured concentrations tested and the corresponding immobilization data were used to estimate the 24- and 48-hour EC_{50} and 95% confidence intervals. The 24-EC_{50} and corresponding 95% confidence intervals were determined by probit analysis. The 48-EC_{50} and corresponding 95% confidence intervals were determined by moving average angle analysis. It appears that the NOEC was estimated by visual inspection of the immobilization data.

Results Synopsis:

24-Hour Values

EC_{50} = 2.8 µg a.i./L 95% confidence intervals = 2.2-3.9 µg a.i./L

48-Hour Values

EC_{50} = 1.5 µg a.i./L 95% confidence intervals = 1.2-1.9 µg a.i./L

NOEC: 0.32 µg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Versar could not verify the EC50 value. The study value was based on survival and reproductive success. Although versar used mean survival data within the computer program Toxanal; the program results were inconclusive and should not be utilized.

14. REVIEWER'S COMMENTS:

No additional comments.

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)- (94.6%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** R107894 **Purity:** 94.6%

3. **CITATION**

Author:	Arthur E. Putt
Title:	R107894 – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions.
Study Completion Date:	April 29, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1075
Sponsor:	Janssen Pharmaceutica N. V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6143
MRID No.:	465960-02

4. **REVIEWED BY:**

   Signature: **Date:** 2/17/06
   David C. Bays, Microbiologist, RASSB, AD (7510C)

5. **APPROVED BY:**

   Signature: **Date:** 2/17/06
   Rick Petrie, Team 3 Leader, RASSB, AD (7510C)
   Kay Montague, Acting Team 2 Leader, RASSB, AD (7510C), for 3/00/06

6. **STUDY PARAMETERS**

   Scientific Name of Test Organism: *Oncorhynchus mykiss*
   Age of Test Organism: Not reported
   Definitive Test Duration: 96 hours
   Study Method: Flow-through
   Type of Concentrations: Nominal and mean-measured

7. **CONCLUSIONS**

Results Synopsis:

96-hour LC50:	1.3 µg a.i./L	95% C.I.:	0.68-2.1 µg a.i./L
NOEC:	0.68 µg a.i./L		

8. ADEQUACY OF THE STUDY

A. **Classification:** Core

B. **Rationale:** Scientifically sound study. Only had minor guideline deviations that did not significantly affect the results of the study.

C. **Repairability:** N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- Fish were not noted as either being or not being from the same class year.
- No statement was made as to the signs of disease 48-hours prior to testing.
- The biomass loading was 0.14 g/L/day, instead of the guideline requirement of 1 g/L/day.
- The pH ranged from 6.9 to 7.4 throughout the study period. Guidelines state that the pH should be between 7.2 and 7.4.
- The temperature varied from 12 to 13 °C, while guidelines state that cold water species should be held and tested at 12°C
- Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm with a fill volume of 6.8 L were used. Guidelines state that the aquaria should be 30 x 60 x 20 cm and have a fill volume of 15 to 30 L of solution.
- Average measured concentrations of the test substance (sum of R107894 and CL 322,250) in the 0.54 and 0.90 µg a.i/L treatment levels were 68 and 76% of nominal, respectively. Guidelines state that test concentrations should remain at least 80 percent of the nominal concentrations throughout the test. The study author noted that the low recoveries in the 0.54 and 0.90 µg a.i/L treatment levels were a function of the calibration of the diluter system and did not have a significant impact on the results of the study.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

DP Barcode: MRID No:

Guideline Criteria	Reported Information
• Species Preferred species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*)	• Rainbow Trout (*Oncorhynchus mykiss*)
Mean Weight • 0.5-5 g	• Mean: 0.77 g (Range: 0.49-1.1 g) (p. 9)
Mean Standard Length • Longest not > 2x shortest	• Mean: 43 mm (Range: 37-46 mm) (p. 9)
Supplier	• Troutlodge, Inc., Sumner, Washington (p. 13)
All fish from same source?	• Yes (p. 13)
All fish from the same year class?	• Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period • Minimum 14 days	• Fish were held in holding tank for 14 days under conditions similar to test. (p. 13)
Wild caught organisms were quarantined for 7 days?	• Not applicable
Were there signs of disease or injury?	• Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	• No sign of mortality 48-hours prior to testing, no other observations provided. (p. 13)
Feeding • No feeding during the study	• Fish were not fed during the exposure period or two days prior to the experiment. (p. 13)
Pretest Mortality • No more than 3% mortality 48 hours prior to testing	• No mortality was observed among the test fish population during the 48-hour period prior to testing. (p. 13)

C. Test System

Guideline Criteria	Reported Information
Source of dilution water • Soft reconstituted water or water from a natural source, not dechlorinated tap water	• Soft water from a 100-m deep bedrock well supplemented with well water supplied by the town of Wareham, Massachusetts. (p. 13)
Does water support test animals without observable signs of stress?	• Yes, freshwater organisms have survived and reproduced for generations in the well water. (p. 14)

Guideline Criteria	Reported Information
Water Temperature • 12 C for cold water species • 17 C or 22 C for warm water species	• Test temperature ranged from 12 to 13 °C (p. 24)
pH • Prefer 7.2 to 7.6	• pH ranged from 6.9 to 7.4 (p. 24)
Dissolved Oxygen • Static: 60% during 1st 48 hrs and 40% during 2nd 48 hrs • Flow-through: 60%	• Dissolved oxygen concentrations were above 75% saturation throughout the study. (p. 24)
Total Hardness • Prefer 40 to 48 mg/L as $CaCO_3$	• 46 to 52 mg/L as $CaCO_3$. (p. 14)
Test Aquaria • Material: Glass or stainless steel • Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm • Fill volume: 15-30 L of solution	• Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm (p. 14 and 16) • Fill Volume: 6.8 L. (p.16)
Type of Dilution System • Must provide reproducible supply of toxicant	• An intermittent-flow proportional diluter was used in the experiment. Measured concentrations of the test substance were consistent between sampling intervals and maintained the expected concentration gradient. (p. 20)
Flow Rate • Consistent flow rate of 5-10 vol/24 hours • Meter systems calibrated before study and checked twice daily during test period	• Constant flow rate at 8.1 solution volume replacements per day. (p. 16) • Diluter system was calibrated prior to test initiation and at test termination. The diluter was monitored daily (flow rates, stock solution consumption) and a visual check was performed twice each day. (p. 16)
Biomass Loading Rate • Static: 0.8 g/L at 17 C, 0.5 g/L at > 17 C • Flow-through: 1 g/L/day	• 0.14 grams of biomass per liter of solution per day. (p. 16)
Photoperiod • 16 hours light, 8 hours dark	• 16 hours light, 8 hours dark (p. 14)
Solvents • Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	• Acetone: 0.10 mL/L (p. 15)

D. Test Design

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Range Finding Test • If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	• Preliminary test conducted • Five test organisms per treatment level • After 96 hours, 100% mortality in the 2.5 µg a.i/L treatment level. Mortality of 20% in the 1.5 µg a.i/L treatment level. No mortality or adverse effects in the remaining treatment levels (0.90, 0.54, and 0.32 µg a.i/L) or the control. (p. 19)
Nominal Concentrations of Definitive Test • Control & 5 treatment levels • Dosage should be 60% of the next highest concentration • Concentrations should be in a geometric series	• Control, solvent control and 5 treatment levels: 0.32, 0.54, 0.90, 1.5, and 2.5 µg a.i./L. (p. 15) • Nominal concentrations were approximately 60% of the next highest concentration. • Concentrations were in a geometric series.
Number of Test Organisms • Minimum 10/level • May be divided among containers	• Ten fish per replicate test aquarium; 2 replicates per treatment (20 fish per treatment level and controls). (p. 16)
Test organisms randomly or impartially assigned to test vessels?	• Fish were impartially selected from the holding tank and placed two at a time in each replicate test aquarium. (p. 16).
Biological observations made every 24 hours?	• Yes (p. 16)
Water Parameter Measurements • Temperature: Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1 C • DO and pH: Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	• Test solution temperature was continuously monitored during the test in one replicate of the solvent control. In the treatment and control tanks, temperature was measured once daily. (p. 17) • Temperatures did not vary more than a degree. (p. 24) • DO and pH were measured once daily in the treatment and control tanks. (p. 17)

Guideline Criteria	Reported Information
	• Prior to initiation, samples taken from replicates of high, medium, low and control treatment levels and analyzed for R107894 and the degradate CL 322,250 (p.17)
Chemical Analysis • Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	• Sample of stock solution analyzed during pre-test period for R107894 and CL 322,250 (p. 17) • One water sample from one replicate of each treatment level and control solutions was collected and analyzed for R107894 and CL 322,250 at test initiation and test termination. (p. 17) • Samples removed from alternate replicates at test initiation and termination (p. 17)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	• Yes (p.3-4)
Percent Recovery of Chemical from Chemical Analysis	• At test termination, measured concentrations of R107894 ranged from 68 to 82% of the nominal concentrations. (p. 20) • At test termination, measured concentrations of CL 322,250 were below the level of detection for all treatments except for the 2.5 µg a.i/L treatment level, which was 14% of nominal R107894 concentration. (p. 20)
Control Mortality • Not more than 10% control organisms may die or show abnormal behavior.	• No mortality was observed in the controls. (p. 27)
Raw data included?	• Yes (p. 24-30)
Signs of toxicity (if any) were described?	• Yes, organisms were noted to be dead, lethargic, or dark in coloration. (p. 27)

Dose Response

Mortality

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Number of Fish at Test Initiation	Number of Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Control	Control	10/10	0/0	0/0	0/0	0/0
Solvent Control	Solvent Control	10/10	0/0	0/0	0/0	0/0
0.32	0.26	10/10	0/0	0/0	0/0	0/0
0.54	0.37	10/10	0/0	0/0	0/0	0/0
0.90	0.68	10/10	0/0	0/0	0/0	0/0
1.5	1.2	10/10	1/0	2/1	3/1	5/2
2.5	2.1	10/10	7/3	10/8	10/9	10/10

Symptoms

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Symptoms			
		24 hour	48 hour	72 hour	96 hour
Control	Control	0	0	0	0
Solvent Control	Solvent Control	0	0	0	0
0.32	0.26	0	0	0	0
0.54	0.37	0	0	0	0
0.90	0.68	0	0	0	0
1.5	1.2	1[a,b] & 1[b]	0	0	0
2.5	2.1	9[c,f] & 1[d]	2[a]	1[a]	0

a Observed to be lethargic
b Observed to be dark in coloration
c Several surviving fish were observed to be lethargic and dark in coloration
d Exhibited partial loss of equilibrium, a dark pigmentation and was at the surface of the test solution.
f Several surviving fish were observed to be lethargic

Statistical Results

Statistical Method: The 24-, 48-, and 72-hour LC_{50}'s were estimated using probit analysis. The 96-hour LC_{50} was estimated using binomial probability. The NOEC was estimated by visual inspection. (p. 28)

Results Synopsis:

24-hour LC_{50}: 2.1 µg a.i./L Confidence (95%) interval: 1.8-3.0 µg a.i./L
48-hour LC_{50}: 1.5 µg a.i./L Confidence (95%) interval: 1.4-1.8 µg a.i./L
72-hour LC_{50}: 1.5 µg a.i./L Confidence (95%) interval: 1.3-1.7 µg a.i./L
96-hour LC_{50}: 1.3 µg a.i./L Confidence (95%) interval: 0.68-2.1 µg a.i./L
96-hour NOEC: 0.68 µg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Not performed.

14. REVIEWER'S COMMENTS:

No additional comments.

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)- (94.6%) (ECONEA Technical)

PC Code No.: 119093

2. **TEST MATERIAL:** R107894 **Purity:** 94.6%

3. **CITATION**

Author:	Arthur E. Putt
Title:	R107894 – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions.
Study Completion Date:	April 22, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1075
Sponsor:	Janssen Pharmaceutica N. V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6142
MRID No.:	465960-03

4. **REVIEWED BY:**

Signature: **Date:** 2/17/06
David C. Bays, Microbiologist, RASSB, AD (7510C)

5. **APPROVED BY:**

Signature: **Date:** 2/17/06
Rick Petrie, Team 3 Leader, RASSB, AD (7510C)
Kay Montagne, Acting Team 2 Leader, RASSB, AD (7510C) 3/20/06

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Leponis macrochiru*
Age of Test Organism: Not reported
Definitive Test Duration: 96 hours
Study Method: Flow-through
Type of Concentrations: Nominal and mean-measured

7. **CONCLUSIONS**

Results Synopsis:

96-hour LC50: 3.2 µg a.i./L 95% C.I.: 2.8-3.7 µg a.i./L

96-hour NOEC: 1.3 µg a.i./L

8. **ADEQUACY OF THE STUDY**

A. Classification: Core

B. Rationale: Scientifically sound study. Only had minor guideline deviations that did not affect the results of the study.

C. **Repairability:** N/A

9. **GUIDELINE DEVIATIONS:**

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- Fish were not noted as either being or not being from the same class year.
- No statement was made as to the signs of disease 48-hours prior to testing.
- Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm with a fill volume of 6.8 L. Guidelines state that the aquaria should be 30 x 60 x 20 cm and have a fill volume of 15 to 30 L of solution.
- The biomass loading was 0.16 g/L/day instead of the guideline requirement of 1 g/L/day.
- The dissolved oxygen level dropped below the 75% guideline requirement in two replicate chambers of the treatments. The study author noted that this deviation did not have a negative impact on the results of the study.
- Test temperatures ranged from 21 to 23 °C. Guidelines state that the test temperature should not vary more than 1 °C.
- The pH during exposure period ranged from 6.9 to 7.6. Guidelines state that the pH should range between 7.2-7.6.
- The hardness ($CaCO_3$) of the test system water ranged from 46-48 mg/L. Guidelines state that the hardness should range from 46-48 mg/L.

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
• **Species** Preferred species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*)	• Bluegill Sunfish (*Lepomis macrochirus*)
Mean Weight • 0.5-5 g	• Mean: 0.84 g (p. 9) • Range: 0.28 to 1.33 g (p.9)
Mean Standard Length • Longest not > 2x shortest	• Mean: 40 mm (p. 9) • Range: 31 to 44 mm (p. 9)
Supplier	• Osage Catfisheries, Osage Beach, Missouri (p. 9)
All fish from same source?	• Yes
All fish from the same year class?	• Not provided.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period • Minimum 14 days	• Fish were held in holding tank for 14 days under conditions similar to test conditions. (p. 13)
Wild caught organisms were quarantined for 7 days?	• Not applicable.
Were there signs of disease or injury?	• Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	• No sign of mortality 48-hours prior to testing, no other observations provided. (p. 13)
Feeding • No feeding during the study	• Fish were not fed during the exposure period or 48 hours prior to the experiment. (p. 13)
Pretest Mortality • No more than 3% mortality 48 hours prior to testing	• No mortality was observed among the test fish population during the 48-hour period prior to testing. (p. 13)

C. Test System

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Source of dilution water • Soft reconstituted water or water from a natural source, not dechlorinated tap water	• Soft water from a 100-m deep bedrock well supplemented with well water supplied by the town of Wareham, Massachusetts. (p. 13)
Does water support test animals without observable signs of stress?	• Yes, freshwater organisms (daphnids) have survived and reproduced for generations in the well water. (p. 14)
Water Temperature • 12 C for cold water species • 17 C or 22 C for warm water species	• Exposure solution temperature ranged from 21 to 23 °C (p. 20)
pH • Prefer 7.2 to 7.6	• pH during exposure period ranged from 6.9 to 7.6 (p. 25)
Dissolved Oxygen • Static: 60% during 1st 48 hrs and 40% during 2nd 48 hrs • Flow-through: 60%	• At 72 hours of exposure, the dissolved oxygen concentration in replicate A of the 3.6 µg a.i./L treatment level was 74%. At 72 and 96 hours of exposure, dissolved oxygen concentrations in replicate B of the 6.0 µg a.i./L treatment level were 68 and 74%. All other replicates were above 75% saturation. (p.20, 25)
Total Hardness • Prefer 40 to 48 mg/L as $CaCO_3$	• 46 to 48 mg/L as $CaCO_3$ (p. 14).
Test Aquaria • Material: Glass or stainless steel • Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm • Fill volume: 15-30 L of solution	• Glass aquaria with silicone sealant (p. 14) • Each glass aquarium measured 30 x 15 x 20 cm. (p. 16) • Fill Volume: 6.8 L. (p. 16)
Type of Dilution System • Must provide reproducible supply of toxicant	• An intermittent-flow proportional diluter was used in the experiment. Measured concentrations of the test substance were consistent between sampling intervals and maintained the expected concentration gradient (p. 20).
Flow Rate • Consistent flow rate of 5-10 vol/24 hours • Meter systems calibrated before study and checked twice daily during test period	• Constant flow rate at 7.9 solution volume replacements per day (p. 16). • Diluter system was calibrated prior to test initiation and at test termination. The diluter was monitored daily (flow rates, stock solution consumption) and a visual check was performed twice each day. (p. 16).
Biomass Loading Rate • Static: 0.8 g/L at 17 C, 0.5 g/L at > 17 C • Flow-through: 1 g/L/day	• 0.16 grams of biomass per liter of solution per day (p. 16).

Photoperiod • 16 hours light, 8 hours dark	• 16 hours light, 8 hours dark (p. 14)
Solvents • Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	• Acetone: 0.10 mL/L (p. 15)

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test • If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	• Two preliminary exposure experiments were conducted. • Five test organisms per treatment level in each experiment. • In the first test, 60% and 100% mortality was observed at treatment levels of 13 and 22 µg a.i./L, respectively. In the second experiment, 100% and 80% mortality was observed at treatment levels of 10 and 6.0 µg a.i./L, respectively. (p. 19).
Nominal Concentrations of Definitive Test • Control & 5 treatment levels • Dosage should be 60% of the next highest concentration • Concentrations should be in a geometric series	• Control, solvent control, and 5 treatment levels: 1.3, 2.2, 3.6, and 10 µg a.i./L. (p. 15) • Nominal concentrations were approximately 60% of the next highest concentration. • Concentrations were in a geometric series.
Number of Test Organisms • Minimum 10/level • May be divided among containers	• Ten fish per replicate test aquarium; 2 replicates per treatment (20 fish per treatment level and controls). (p. 16)
Test organisms randomly or impartially assigned to test vessels?	• Fish were impartially selected from the holding tank and placed two at a time in each replicate test aquarium. (p. 16).
Biological observations made every 24 hours?	• Yes (p. 16)
Water Parameter Measurements • Temperature: Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1 C • DO and pH: Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	• Test solution temperature was continuously monitored during the test in one replicate of the solvent control. In the treatment and control tanks, temperature was measured once daily. (p. 17) • Temperatures ranged from 21 to 23 °C. (p. 20) • DO and pH were measured once daily in the treatment and control tanks. (p. 17)

Guideline Criteria	Reported Information
Chemical Analysis • Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	• Twice prior to initiation, samples taken from alternate replicates of high, medium, low, and control treatment levels and analyzed for R107894 and the degradate CL 322,250 (p.17) • Sample of stock solution analyzed during pre-test period for R107894 and CL 322,250 (p. 17) • One water sample from one replicate of each treatment level and control solutions was collected and analyzed for R107894 and CL 322,250 at test initiation and test termination. (p. 17) • Samples removed from alternate replicates and initiation and termination (p. 17)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	• Yes (p.3-4)
Percent Recovery of Chemical from Chemical Analysis	• At test termination, measured concentrations of R107894 ranged from 57 to 69% of the nominal concentrations. (p. 20) • At test termination, measured concentrations of CL 322,250 ranged from 21 to 26% of the nominal R107894 concentrations. (p. 21)
Control Mortality • Not more than 10% control organisms may die or show abnormal behavior.	• No mortality was observed in the controls. (p. 21)
Raw data included?	• Yes.
Signs of toxicity (if any) were described?	• Yes, organisms were noted to be dead, lethargic, or dark in coloration. (p. 28)

Dose Response

Mortality

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Number of Fish at Test Initiation (Rep A / Rep B)	Number of Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Control	Control	10/10	0/0	0/0	0/0	0/0
Solvent Control	Solvent Control	10/10	0/0	0/0	0/0	0/0
1.3	0.80	10/10	0/0	0/0	0/0	0/0
2.2	1.3	10/10	0/0	0/0	0/0	0/0
3.6	2.2	10/10	0/0	0/0	1/0	2/0
6.0	4.1	10/10	0/0	2/1	7/3	8/8
10	6.8	10/10	8/6	10/10	10/10	10/10

Symptoms

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Symptoms			
		24 hour	48 hour	72 hour	96 hour
Control	Control	0	0	0	0
Solvent Control	Solvent Control	0	0	0	0
1.3	0.80	0	0	0	0
2.2	1.3	0	0	0	0
3.6	2.2	0	0	0	0
6.0	4.1	0	0	8[b] & 2[c]	3[b] & 1[c]
10	6.8	6[a]	0	0	0

a Observed to be lethargic
b Several surviving fish were observed to be lethargic.
c Observed to be lethargic and dark in coloration

Statistical Results

Statistical Method: The 24- and 48-hour LC_{50}'s were estimated using binomial probability. The 72- and 96-hour LC_{50}'s were estimated using probit analysis. The NOEC was estimated by visual inspection.

Results Synopsis:

24-hour LC_{50}:	6.1 µg a.i./L	Confidence (95%) interval:	4.1 µg a.i./L (upper confidence interval could not be calculated)
48-hour LC_{50}:	4.9 µg a.i./L	Confidence (95%) interval:	4.1-6.8 µg a.i./L
72-hour LC_{50}:	3.9 µg a.i./L	Confidence (95%) interval:	3.3-4.5 µg a.i./L
96-hour LC_{50}:	3.2 µg a.i./L	Confidence (95%) interval:	2.8-3.7 µg a.i./L
96-hour NOEC:	1.3 µg a.i./L		

13. VERIFICATION OF STATISTICAL RESULTS

Not performed.

14. REVIEWER'S COMMENTS:

No additional comments.

DATA EVALUATION RECORD
DAPHNID CHRONIC TOXICITY TEST
GUIDELINE OPPTS 850.1300

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5 (trifluoromethyl)- (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** R107894 **Purity:** 94.6%
   Lot or Batch No.: ACI2649-8

3. **CITATION**

   Authors: Mark A. Cafarella
   Title: R107894 -- Full Life-Cycle Toxicity Test With Water Fleas, *Daphnia magna*, Under Flow-Through Conditions
   Study Completion Date: June 27, 2005
   Laboratory: Springborn Smither Laboratories
   790 Main Street
   Wareham, Massachusetts 02571-1037
   Sponsor: Janssen Pharmaceutica N.V.
   Plant and Material Protection Division
   Turnhoutseweg 30
   B-2340 Beerse, Belgium
   Laboratory Report ID: Springborn Smithers Study No.: 13751.6145
   Sponsor Protocol/Project No. AGR 922
   MRID No.: 465960-04

4. **REVIEWED BY:**

   Signature: **Date: 10/12/06**
   David C. Bays, Microbiologist, RASSB, AD (7510C)

5. **APPROVED BY:**

   Signature: **Date: 10/12/06**
   Rick Petrie, Team 3 Leader, RASSB, AD (7510C)
   Kay Montague, Acting Team 2 Leader, RASSB, AD (7510C)

6. **STUDY PARAMETERS**

   Scientific Name of Test Organism: *Daphnia magna*
   Age of Test Organism: <24 hours old
   Definitive Test Duration: 21 days
   Study Method: Flow-Through
   Type of Concentrations: Mean-measured

7. **CONCLUSIONS**

Results Synopsis: NOEC = 0.20 µg ai/L
LOEC = 0.57 µg ai/L
MATC = 0.34 µg ai/L
EC50 (95% CI) = 0.78 µg ai/L (0.57 to 1.0 µg ai/L)

8. ADEQUACY OF THE STUDY

A. **Classification:** Core

B. **Rationale:** Scientifically acceptable study.

C. **Repairability:** Suitable information regarding pre-test culture conditions and aeration was submitted so study was upgraded to core. (See MRID 469179-01 and explanations below)

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.1300:

- The length of the acclimation period was not reported. **(Since the adults were maintained in continuous culture and maintained in conditions similar to those reported, the length of acclimation period was not pertinent)**
- During culture, daphnids were fed 2.0 mL of a unicellular green algae and 0.5 mL of YCT suspension per test vessel once daily. During definitive exposure, daphnids were fed the same diet at rates of 3.0 mL of algal suspension and 1.0 mL YCT suspension per test vessel, three times daily. The guidelines state that during the definitive test, daphnids should be fed same diet and at the same frequency as the cultures. **(Cultures were maintained under static conditions, however, this study was conducted under flow-through test conditions in a effort to maintain suitable concentrations of toxicant. Due to the nature of flow-through testing, the feeding regime is modified to allow accessibility to adequate food source)**
- A comparison of the flow rates from each test chamber was not reported. **(In this study, the flow-splitting accuracy was within 10% of the targeted delivery)**
- The test was conducted according to GLPs; however, it was not reported if the equipment and chambers were cleaned prior to each use. **(All exposure systems (diluters, test chambers, etc) are thoroughly cleaned prior to each use)**
- It was not reported whether the test water was aerated before addition of the test substance. **(Dilution water was aerated prior to delivery to the exposure system)**
- It was not reported whether the controls daphnids did not produce any ephippia. **(No ephippia were produced during the exposure, since no males were used in the study and they are the only ones that can produce ephippia)**

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. **Test Organisms**

Guideline Criteria	Reported Information

Species • *Daphnia magna* • *D. pulex*	• *Daphnia magna* were used. (p. 9)
Life Stage • First instar, #24 hours old.	• All daphnia were <24 hours old. (p. 9)
Source • Daphnids should be cultured at the test facility and originate from same culture population.	• The daphnia used were obtained from laboratory cultures maintained at Springborn Smithers. (p. 14)
Culturing: • Source of initial stock and culturing techniques described. • Do not use daphnids if: • - Cultures contain ephippia. • - Adults in cultures do not produce young before day 12. • - More than 20% of the culture stock dies during the 2 days preceding the test. • - Adults in the culture do not produce an average of at least three young per day over the 7-day period prior to test. • - Daphnids have been used in any portion of a previous test, either in a treatment or in a control.	• The daphnia used were obtained from laboratory cultures maintained at Springborn Smithers. (p. 14) • The adult daphnids used to produce offspring did not contain ephippia, produced offspring prior to being 12 days old, had a survival rate 48 hours prior to the test of 100%, produced an average of 3.6 offspring per female per day seven days prior to test initiation, and were not used in any portion of a previous test. (p. 14)
Acclimation • Acclimate at least 48 hours prior to start of test. • Maintain in 100% dilution water at test conditions (temperature, diet, background colors, and light intensity). • Should be fed same food as used during definitive test	• Adults were maintained in continuous culture and maintained in conditions similar to those reported, the length of acclimation period as not pertinent. • Daphnid cultures are maintained in water from the same source as the dilution water utilized in this study and have successfully survived and reproduced over multiple generations. (p. 15)
Feeding • During test, daphnids should be fed same diet and at same frequency as cultures. • Suggested rates: 5 to 7 mg/L of dilution water or test solution (automatic); 15 mg (dry weight)/L (manual).	• During culture, daphnids were fed 2.0 mL of a unicellular green algae (*Ankistrodesmus falcatus*, approx. 4 x 10^5 cells/mL of algae) and 0.5 mL of YCT suspension (yeast, cereal leaves, and digested flake fish food) per test vessel once daily. During definitive exposure, daphnids were fed the same diet at rates of 3.0 mL of algal suspension and 1.0 mL YCT suspension per test vessel, three times daily. (p. 14)

B. Test System

Guideline Criteria	Reported Information

System • Static-renewal: dilution water completely replaced at least once every 3 days. • Flow-through: • - Calibrate system before each test. • - Check general operation at least twice during test. • - 24-hour flow through a test chamber should equal at least 5x volume of chamber. • - Flow rate should not vary by more than 10% from one chamber to another.	• The diluter system was calibrated prior to test initiation and confirmed at test termination by measuring delivery volumes of stock solutions. The function of the diluter system was monitored daily and a visual check of the system's operation was performed twice daily. (p. 17) • Test solutions were delivered to the exposure vessels at an approximate rate of 6 test vessel volumes per 24-hour period. (p. 17) • Flow splitting accuracy was within 10% of the targeted delivery
Dilution Water • Surface or ground water, reconstituted water, (deionized) water, or dechlorinated tap water acceptable. • Water quality parameters (maximum): • Particulates 20 mg/L • TOC 2 mg/L or COD 5 mg/L • Un-ionizable ammonia 20 g/L • Residual chlorine <3 µg/L • Total organophosphorus pesticides 50 ng/L • Total organochlorine pesticides plus PCBs (50 ng/L) or organic chlorine 25 ng/L • Water quality should be tested at least twice per year. • If diluent is groundwater or surface water, conductivity and TOC or COD should be measured.	• Culture and test dilution water were prepared by fortifying well water according to the formula for hard water (U.S. EPA, 1975) and filtering it through an Amberlite XAD-7 resin column to remove an organic contaminants. (p. 15) • The water prepared during the definitive exposure was characterized as having a total hardness of 180 to 190 mg/L as $CaCO_3$, total alkalinity of 120 to 130 mg/L as $CaCO_3$, a pH of 8.1 to 8.3, a dissolved oxygen concentration of 10.2 to 11.2 mg/L, and a specific conductivity of 500 µmhos/cm. (p. 15) • Water quality parameters were measured on each batch of fortified water prior to use. (p. 15) • The total organic carbon (TOC) was measured to be 0.25 mg/L and particulate matter was 0.50 mg/L. (p. 15)
Photoperiod • 16-hr light/8-hr dark	• The test area was illuminated with fluorescent bulbs at an intensity of 300-400 lux and a photoperiod of 16 hours light/8 hours darkness. Sudden transitions from light to dark or vice versa were avoided. (p. 16)

Test Chambers • 250-mL beakers or other suitable containers. • Loosely covered to reduce loss of test solution or dilution water due to evaporation and to minimize entry of dust or other particulates. • Test equipment and test chambers should be cleaned before each use using good laboratory practices. • For flow-through tests: daphnids can be in glass or stainless steel containers with stainless steel or nylon bottoms suspended in test chamber to ensure test solution flows regularly into and out of containers and daphnids are always submerged in at least 5 cm of test solution.	• Test vessels were glass battery jars having a total volume capacity of 1.6L. (p. 17) • Test vessels were loosely covered with plastic during the study. (p. 17) • The test was conducted according to GLPs; and equipment and chambers were cleaned prior to each use. • Exposure solutions drained from each vessel through two 2-cm holes approximately 15 cm from the bottom of the jars that maintained the test solution volume at 1.4 L. The drain holes were covered with a Nitex® 40-mesh screen to prevent loss of daphnids. (p. 17)
Temperature • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations. • 20 ± 1EC	• Temperature was measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). In addition, the temperature was measured daily in one vessel of each test concentration and the controls. (p. 19) • Temperature range was 20-21°C. (p. 31)
Dissolved Oxygen • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations. • Between 60 and 105 percent saturation. • Aeration should be done before addition of test substance.	• Dissolved oxygen was measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). In addition, the dissolved oxygen was measured daily in one vessel of each test concentration and the comrols. (p. 19) • Dissolved oxygen values reported were 5.3 to 9.2 mg/L (60% to 101% saturation). (p. 24) • Dilution water was aerated prior to delivery to the exposure system.
pH • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations.	• The pH was measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). In addition, the pH was measured daily in one vessel of each test concentration and the controls (p. 19).
Solvents and Carriers • Concentration of carrier #0.1 mL/L. • Triethylene glycol and dimethyl formamide preferred solvents, but acetone or ethanol can be used if necessary.	• Acetone concentration at 0.1 mL/L. (p. 19) • A 0.035 mg ai/mL stock solution was prepared with 0.0093 g (0.0088 g as ai) of R107894 in a 250-mL volumetric flask and brought to volume with acetone. (p. 16)

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Should be conducted to establish test solution concentrations in definitive test. • Exposure to a series of widely spaced concentrations of the test chemical (e.g., 1, 10, 100 mg/L), usually under static conditions. • Minimum of five daphnids should be exposed to each concentration of test substance. • Exposure period may be shortened if suitable data can be obtained in less time. • No replicates required and nominal concentrations of chemical acceptable.	• A range-finding test was conducted under flow-through conditions exposing daphnids (<25 hours old) to nominal R107894 treatment levels of 0.44, 0.88, 1.8, 3.5, 7.0 µg ai/L and a dilution water control. (p. 23) • Two replicate vessels (10 daphnids/vessel) were exposed over 20 days. (p. 23) • Survival of 80, 95, 65, 25, and 0% was observed among daphinds exposed to the 0.44, 0.88, 1.8, 3.5, and 7.0 µg ai/L treatment levels, respectively. (p. 23)
Doses • Five or more concentration in a geometric series with a 1.5 to 2.0 progression (e.g., 2, 4, 8, 16, 32, and 64 mg/L).	• Based on preliminary exposures, the nominal concentrations of 0.22, 0.44, 0.88, 1.8 and 3.5 µg ai/L were selected for the definitive study with a 2x progression. (p. 23)
Test Substance Concentration • At minimum, concentration of test chemical should be measured in each chamber before the test and on days 7, 14, and 21 of the test, and in at least one appropriate chamber whenever a malfunction is detected. • Concentrations of test substance in replicate test chambers should not vary more than ± 20%.	• Test substance concentrations were measured prior to test initiation and at days 0, 7, 14 and 21 during the in-life portion of the test. (pp. 24, 32) • The nominal test concentrations were 0.22, 0.44, 0.88, 1.8, and 3.5 µg ai/L (p. 16). The mean measured concentrations were determined to be 0.13, 0.20, 0.57, 1.0, and 2.0 µg ai/L. (pp. 24, 32)
Controls X Controls should consist of same dilution water, conditions, and procedures, and daphnids. X Negative and/or solvent	• A dilution water and solvent control were run under the same conditions as the five concentrations of the test substance. (pp. 15, 16)
Replicates Per Dose • Equal number of daphnids in 2 or more replicates per dose (flow-through) • One daphnid each in 10 or more replicates per dose (static-renewal).	• The test concentration and controls contained twenty *Daphnia magna* (10 organisms per replicate test vessel). (p. 18)

Number of Organisms: • Minimum of 20 daphnids per concentration (flow-through). • Minimum of 10 daphnids per concentration (static-renewal). • Test organisms randomly or impartially placed in the test chambers. • Loading should not exceed 40 daphnids per liter of test solution in static-renewal system. • Loading in flow-through test varies depending on flow rate of test solution.	• The test concentration and controls contained twenty *Daphnia magna* (10 organisms per replicate test vessel). (p. 18) • The daphnids were impartially added, two at a time to each intermediate vessel, until each vessel contained two organisms. This was repeated until each intermediate vessel contained ten organisms. The daphnids were then introduced into the replicate exposure vessels by impartially selecting one of the unlabeled intermediate vessels containing ten organisms and gently pipetting them one at time under the surface of the test solution. (p. 18)
Duration of Test • 21 days	• Daphnids were observed for 21 days. (p. 18)
Observation of Daphnids • Daphnids in the test chambers observed on day 21 of the test. • Offspring should be counted and removed from the test chambers every 2 or 3 days. • Abnormal behavior or appearance reported.	• The number of immobilized offspring and adult daphnids and observations of abnormal behavior were recorded on days 0, 2, 4, 7, 11, 13, 14, 15, 18, 19, 20 and 21. (p. 18) • Assessment of the offspring was determined on day 7 and three times per week through day 21. (p. 18)
Test Endpoints Measured • Number of daphnids immobilized (EC_{50} values and 95% C.I.) • Number of young per adult. • MATC determined for most sensitive endpoint.	• The mean measured concentrations tested and the corresponding data for immobilization and reproduction derived from the definitive toxicity test were used to estimate the 21-day EC50 and 95% CI. (p. 22) • The cumulative number of offspring per female in each vessel was determined by dividing the number of counted offspring by the number of surviving female daphnids. (p. 21) • The MATC was calculated based on the LOEC and NOEC. The determination of these levels was based on the most sensitive of the performance criteria evaluated, reproduction and growth. (p. 22)
Growth • Determined by measuring total body length or dry weight (both preferred).	• At test termination, the total body length and dry weight of each surviving adult daphnid was measured. (p. 19)

Validity of Test Test is only valid if: • Less than 20% of the control should be immobilized, stressed, or diseased at the end of the study. • Each control daphnid should have produced at least 60 young after 21 days. • The controls should not produce any ephippia.	• After 21 days of exposure, survival among the control and solvent control organisms both averaged 100%. (p. 26) • The cumulative number of offspring released by each female organism of the control and solvent control organisms was 164 and 174 offspring, respectively. (p. 25) • No ephippia were produced during the exposure period

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes (pp. 3, 4)
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	• Yes (cover page and p. 9)
Growth of the daphnids determined by total body length or body weight?	• Yes (p. 19)
Source of test material, lot number, composition, known chemical and physical properties, and any carriers or other additives used and their concentrations reported?	• Yes (pp. 9, 13, 16)
Source of the dilution water, its chemical characteristics (e.g. conductivity, hardness, pH), and a description of any pretreatment reported?	• Yes (p. 15)
Detailed information about the daphnids provided?	• Yes (p. 14)
Description of the test chambers provided?	• Yes (pp. 15-17)
Concentration of the test substance in the test chambers at the designated times provided?	• Yes (pp. 24, 25)
Number and percentage of organisms that showed any adverse effect reported?	• Yes (p. 26)
Cumulative adult and offspring immobilization values, progeny produced, the time to first brood, the number offspring per adult, and growth of surviving adults measured?	• Yes (pp. 25-27)
All chemical analysis (of water quality) and test substance concentrations, including methods, method validation, and reagents reported?	• Yes (pp. 20, 31, 32, App. A – p. 54)

Data records of the culture, acclimation, and test temperature provided?	• Data records for the water temperature during the test were provided (p. 31). Continuous culture conditions were sustained.
Deviations from the test guideline provided and anything unusual about test (e.g., dilution failure, temperature fluctuations) reported?	• Yes (p. 28).
MATC reported and statistical methods employed reported?	• Yes (pp. 21, 22).
Concentration-response curves utilizing average test substance concentration and adult immobilization data at 21 days provided?	• Graphs were provided. (p. 38)
EC50 value based on adult immobilization calculated using the average measured concentration of the test substance?	• Yes (p. 22)
Raw data included:	• Yes, some raw data were provided for each replicate for length and dry weight; however, only mean values per concentration level were provided for percent survival and number of offspring produced. (p. 78-81)
Statistical methods reported:	• Yes (pp. 20-22)

Dose Response

Nominal Concentration (%)	Mean Concentration (mg/L)	Survival (%)	Mean Cumulative No. Offspring Produced Over 21 Days	Mean Total Body Length at Day 21 (mm)	Mean Total Dry Weight at Day 21 (mg)
Control	<0.072	100	164	4.79	1.08
Solvent Control	<0.072	100	174	4.77	1.16
0.22	0.13	95	176	4.80	1.11
0.44	0.20	100	165	4.83	1.15
0.88	0.57	100	135	4.54	0.87
1.8	1.0	5	34	4.10[a]	0.51[a]
3.5	2.0	0	1	NA[b]	NA[b]

a based on measurements provided for one surviving organism in one replicate of the dose.
b No surviving organisms.

Statistical Results

Statistical Method: The following statistical procedures were utilized:

- Significant differences in percent survival were evaluated after transformation of the data.
- Shapiro Wilk's Test for normality was used to compare the observed sample distribution with a normal distribution for survival, reproduction and growth.
- Bartlett's Test was used as a check on the assumption of homogeneity of variance, data for survival, reproduction, length and weight.
- A t-test was used to compare the survival, reproduction and growth of the control to that of the solvent control.
- If the data passed the two tests for normality and homogeneity of variance, then a parametric method was used to evaluate the results of the life-cycle test. If the data failed the tests for either normality or homogeneity of variance, then a non-parametric method (Dunn's or Steel's One Many Rank Test) was used to evaluate the results of the life-cycle test.
- The MATC was determined based on the limits set by the LOEC and the NOEC. The determination of these levels is based on the most sensitive of the performance criteria evaluated.
- The mean-measured concentrations tested and the corresponding data for immobilizing and reproduction derived from the definitive toxicity test were used to estimate the 21-day median effect concentration (EC50) and the corresponding 95% CI. Three methods were available: moving average angle analysis, probit analysis, and binomial probability.

Results Synopsis:

NOEC = 0.20 µg ai/L
LOEC = 0.57 µg ai/L
MATC = 0.34 µg ai/L
EC50 (95% CI) = 0.78 µg ai/L (0.57 to 1.0 µg ai/L)

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: A parametric t-test was used to determine if there was a statistical difference between the control and solvent control data. If no difference was observed, the control data were pooled for further statistical analyses. Pooled control data were compared to treatment means. Data were first analyzed for normality and homogeneity of variances. If the data were not found to be normally distributed or the variances not homogenous, the Wilcoxan Rank Sum Test or the Steel's Many One Rank Test was used to compare control and treatment means and to determine the NOEC. Otherwise, Dunnett's Test was used to determine the NOEC. The EC50 was determined using the Toxanal program that provides results from three statistical methods: the binomial method, the moving average method, and the probit method.

Verified Results Synopsis:

EC50
Versar could not verify the EC50 value. The study value was based on survival and reproductive success. Although versar used mean survival data within the computer program Toxanal; the program results were inconclusive and should not be utilized. Individual organism reproductive successes were not provided in order to run EPA's Linear Interpolation program, as an alternative.

NOEC/LOEC
Based on both dry weight and length:

NOEC = 0.44 µg ai/L
LOEC = 0.88 µg ai/L

**Data for the 1.8 ug a.i./L and 3.5 ug a.i./L were not included in verification of the NOEC and LOEC. There was a statistical significance that arises due to the fact that a majority of the organisms were dead and not included in the data sets for length as well as dry weight.

MATC
Versar calculated the MATC by taking the geometric mean of the NOEC and LOEC. The MATC was determined to be 0.40 ug a.i./L and matched the result stated in the study report.

MATC = 0.62 µg ai/L

14. REVIEWER'S COMMENTS: Versar's verified NOEC and LOEC do not agree with study results. This can be attributed to a number of factors including the use of differing computer programs and statistical techniques.

EC50 Based on Survival-TOXANAL-Inconclusive

45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	29.35302	88.50114	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.816341
95 PERCENT CONFIDENCE LIMITS =-21.27784 AND 30.91052

LC50 = 1.199483
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .6535945
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
****
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
?

Length- NOEC/LOEC-TOXSTAT

Length
File: R Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	4.776	4.776		
2	0.22	4.800	4.800	-0.834	
3	0.44	4.830	4.830	-1.919	
4	0.88	4.542	4.542	8.347	*

Bonferroni t table value = 2.16 (1 Tailed Value, P=0.05, df=90,3)

Length
File: R Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

	NUM OF	Minimum Sig Diff	% of	DIFFERENCE

GROUP	IDENTIFICATION	REPS	(IN ORIG. UNITS)	CONTROL	FROM CONTROL
1	GRPS 1&2 POOLED	40			
2	0.22	19	0.062	1.3	-0.024
3	0.44	20	0.061	1.3	-0.054
4	0.88	20	0.061	1.3	0.234

Dry Weight- NOEC/LOEC

Dry Weight
File: R2 Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	1.122	1.122		
2	0.22	1.115	1.115	0.220	
3	0.44	1.154	1.154	-0.909	
4	0.88	0.865	0.865	7.419	*

Bonferroni t table value = 2.16 (1 Tailed Value, P=0.05, df=90,3)

Dry Weight
File: r2 Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	40			
2	0.22	19	0.076	6.8	0.008
3	0.44	20	0.075	6.7	-0.032
4	0.88	20	0.075	6.7	0.257

DATA EVALUATION RECORD
DAPHNID CHRONIC TOXICITY TEST
GUIDELINE OPPTS 850.1300

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5 (trifluoromethlıyl)-(93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** R107894 **Purity:** 94.6%
   Lot or Batch No.: AC12649-8

3. **CITATION**

   Authors: Mark A. Cafarella
   Title: R107894 – Full Life-Cycle Toxicity Test With Water Fleas, *Daphnia magna*, Under Flow-Through Conditions
   Study Completion Date: June 27, 2005
   Laboratory: Springborn Smither Laboratories
   790 Main Street
   Wareham, Massachusetts 02571-1037
   Sponsor: Janssen Pharmaceutica N.V.
   Plant and Material Protection Division
   Turnhoutseweg 30
   B-2340 Beerse, Belgium
   Laboratory Report ID: Springborn Smithers Study No.: 13751.6145
   Sponsor Protocol/Project No. AGR 922
   MRID No.: 465960-04

4. **REVIEWED BY:**

   Signature: 2/17/06
   David C. Bays, Microbiologist, RASSB, AD (7510C) **Date: 2/17/06**

5. **APPROVED BY:**

   Signature: **Date: 2/17/06** 3/20/06
   Rick Petrie, Team 3 Leader, RASSB, AD (7510C)
   Kay Montague, Acting Team 2 Leader, RASSB, AD (7510C)

6. **STUDY PARAMETERS**

   Scientific Name of Test Organism: *Daphnia magna*
   Age of Test Organism: <24 hours old
   Definitive Test Duration: 21 days
   Study **Method:** Flow-Through
   Type of Concentrations: Mean-measured

7. **CONCLUSIONS**

Results Synopsis: NOEC = 0.20 µg ai/L
LOEC = 0.57 µg ai/L
MATC = 0.34 µg ai/L
EC50 (95% CI) = 0.78 µg ai/L (0.57 to 1.0 µg ai/L)

Verified Results Synopsis: NOEC = 0.44 µg ai/L
LOEC = 0.88 µg ai/L
MATC = 0.62 µg ai/L

8. ADEQUACY OF THE STUDY

A. Classification: Invalid

B. Rationale: Pre-test culture conditions, including presence of ehippia, were not reported. Presence of ephippia invalidates the test; additionally, information on aeration was not provided. This information must be submitted in order to upgrade this study to acceptable.

C. Repairability: If suitable information regarding pre-test culture conditions and aeration is submitted, study may be upgraded.

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.1300:

- The length of the acclimation period was not reported.
- During culture, daphnids were fed 2.0 mL of a unicellular green algae and 0.5 mL of YCT suspension per test vessel once daily. During definitive exposure, daphnids were fed the same diet at rates of 3.0 mL of algal suspension and 1.0 mL YCT suspension per test vessel, three times daily. The guidelines state that during the definitive test, daphnids should be fed same diet and at the same frequency as the cultures.
- A comparison of the flow rates from each test chamber was not reported.
- The test was conducted according to GLPs; however, it was not reported if the equipment and chambers were cleaned prior to each use.
- It was not reported whether the test water was aerated before addition of the test substance.
- It was not reported whether the controls daphnids did not produce any ephippia.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • *Daphnia magna* • *D. pulex*	• *Daphnia magna* were used. (p. 9)
Life Stage • First instar, ≤24 hours old.	• All daphnia were <24 hours old. (p. 9)

Source • Daphnids should be cultured at the test facility and originate from same culture population.	• The daphnia used were obtained from laboratory cultures maintained at Springborn Smithers. (p. 14)
Culturing: • Source of initial stock and culturing techniques described. • Do not use daphnids if: • - Cultures contain ephippia. • - Adults in cultures do not produce young before day 12. • - More than 20% of the culture stock dies during the 2 days preceding the test. • - Adults in the culture do not produce an average of at least three young per day over the 7-day period prior to test. • - Daphnids have been used in any portion of a previous test, either in a treatment or in a control.	• The daphnia used were obtained from laboratory cultures maintained at Springborn Smithers. (p. 14) • The adult daphnids used to produce offspring did not contain ephippia, produced offspring prior to being 12 days old, had a survival rate 48 hours prior to the test of 100%, produced an average of 3.6 offspring per female per day seven days prior to test initiation, and were not used in any portion of a previous test. (p. 14)
Acclimation • Acclimate at least 48 hours prior to start of test. • Maintain in 100% dilution water at test conditions (temperature, diet, background colors, and light intensity). • Should be fed same food as used during definitive test	• The length of the acclimation period was not reported. • Daphind cultures are maintained in water from the same source as the dilution water utilized in this study and have successfully survived and reproduced over multiple generations. (p. 15)
Feeding • During test, daphnids should be fed same diet and at same frequency as cultures. • Suggested rates: 5 to 7 mg/L of dilution water or test solution (automatic); 15 mg (dry weight)/L (manual).	• During culture, daphnids were fed 2.0 mL of a unicellular green algae (*Ankistrodesmus falcatus*, approx. 4 x 10^7 cells/mL of algae) and 0.5 mL of YCT suspension (yeast, cereal leaves, and digested flake fish food) per test vessel once daily. During definitive exposure, daphnids were fed the same diet at rates of 3.0 mL of algal suspension and 1.0 mL YCT suspension per test vessel, three times daily. (p. 14)

B. Test System

Guideline Criteria	Reported Information

System • Static-renewal: dilution water completely replaced at least once every 3 days. • Flow-through: • - Calibrate system before each test. • - Check general operation at least twice during test. • - 24-hour flow through a test chamber should equal at least 5x volume of chamber. • - Flow rate should not vary by more than 10% from one chamber to another.	• The diluter system was calibrated prior to test initiation and confirmed at test termination by measuring delivery volumes of stock solutions. The function of the diluter system was monitored daily and a visual check of the system's operation was performed twice daily. (p. 17) • Test solutions were delivered to the exposure vessels at an approximate rate of 6 test vessel volumes per 24-hour period. (p. 17) • Comparison of flow rates from each chamber was not reported.
Dilution Water • Surface or ground water, reconstituted water, (deionized) water, or dechlorinated tap water acceptable. • Water quality parameters (maximum): • Particulates 20 mg/L • TOC 2 mg/L or COD 5 mg/L • Un-ionizable ammonia 20 g/L • Residual chlorine <3 µg/L • Total organophosphorus pesticides 50 ng/L • Total organochlorine pesticides plus PCBs (50 ng/L) or organic chlorine 25 ng/L • Water quality should be tested at least twice per year. • If diluent is groundwater or surface water, conductivity and TOC or COD should be measured.	• Culture and test dilution water were prepared by fortifying well water according to the formula for hard water (U.S. EPA, 1975) and filtering it through an Amberlite XAD-7 resin column to remove an organic contaminants. (p. 15) • The water prepared during the definitive exposure was characterized as having a total hardness of 180 to 190 mg/L as $CaCO_3$, total alkalinity of 120 to 130 mg/L as $CaCO_3$, a pH of 8.1 to 8.3, a dissolved oxygen concentration of 10.2 to 11.2 mg/L, and a specific conductivity of 500 µmhos/cm. (p. 15) • Water quality parameters were measured on each batch of fortified water prior to use. (p. 15) • The total organic carbon (TOC) was measured to be 0.25 mg/L and particulate matter was 0.50 mg/L. (p. 15)
Photoperiod • 16-hr light/8-hr dark	• The test area was illuminated with fluorescent bulbs at an intensity of 300-400 lux and a photoperiod of 16 hours light/8 hours darkness. Sudden transitions from light to dark or vice versa were avoided. (p. 16)

Test Chambers • 250-mL beakers or other suitable containers. • Loosely covered to reduce loss of test solution or dilution water due to evaporation and to minimize entry of dust or other particulates. • Test equipment and test chambers should be cleaned before each use using good laboratory practices. • For flow-through tests: daphnids can be in glass or stainless steel containers with stainless steel or nylon bottoms suspended in test chamber to ensure test solution flows regularly into and out of containers and daphnids are always submerged in at least 5 cm of test solution.	• Test vessels were glass battery jars having a total volume capacity of 1.6L. (p. 17) • Test vessels were loosely covered with plastic during the study. (p. 17) • The test was conducted according to GLPs; however, it was not reported if the equipment and chambers were cleaned prior to each use. • Exposure solutions drained from each vessel through two 2-cm holes approximately 15 cm from the bottom of the jars that maintained the test solution volume at 1.4 L. The drain holes were covered with a Nitex® 40-mesh screen to prevent loss of daphnids. (p. 17)
Temperature • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations. • 20 ± 1°C	• Temperature was measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). In addition, the temperature was measured daily in one vessel of each test concentration and the controls. (p. 19) • Temperature range was 20-21°C. (p. 31)
Dissolved Oxygen • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations. • Between 60 and 105 percent saturation. • Aeration should be done before addition of test substance.	• Dissolved oxygen was measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). In addition, the dissolved oxygen was measured daily in one vessel of each test concentration and the controls. (p. 19) • Dissolved oxygen values reported were 5.3 to 9.2 mg/L (60% to 101% saturation). (p. 24) • It was not reported whether the water was aerated before addition of the test substance.
pH • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations.	• The pH was measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). In addition, the pH was measured daily in one vessel of each test concentration and the controls (p. 19).
Solvents and Carriers • Concentration of carrier ≤0.1 mL/L. • Triethylene glycol and dimethyl formamide preferred solvents, but acetone or ethanol can be used if necessary.	• Acetone concentration at 0.1 mL/L. (p. 19) • A 0.035 mg ai/mL stock solution was prepared with 0.0093 g (0.0088 g as ai) of R107894 in a 250-mL volumetric flask and brought to volume with acetone. (p. 16)

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Should be conducted to establish test solution concentrations in definitive test. • Exposure to a series of widely spaced concentrations of the test chemical (e.g., 1, 10, 100 mg/L), usually under static conditions. • Minimum of five daphnids should be exposed to each concentration of test substance. • Exposure period may be shortened if suitable data can be obtained in less time. • No replicates required and nominal concentrations of chemical acceptable.	• A range-finding test was conducted under flow-through conditions exposing daphnids (<25 hours old) to nominal R107894 treatment levels of 0.44, 0.88, 1.8, 3.5, 7.0 µg ai/L and a dilution water control. (p. 23) • Two replicate vessels (10 daphnids/vessel) were exposed over 20 days. (p. 23) • Survival of 80, 95, 65, 25, and 0% was observed among daphinds exposed to the 0.44, 0.88, 1.8, 3.5, and 7.0 µg ai/L treatment levels, respectively. (p. 23)
Doses • Five or more concentration in a geometric series with a 1.5 to 2.0 progression (e.g., 2, 4, 8, 16, 32, and 64 mg/L).	• Based on preliminary exposures, the nominal concentrations of 0.22, 0.44, 0.88, 1.8 and 3.5 µg ai/L were selected for the definitive study with a 2x progression. (p. 23)
Test Substance Concentration • At minimum, concentration of test chemical should be measured in each chamber before the test and on days 7, 14, and 21 of the test, and in at least one appropriate chamber whenever a malfunction is detected. • Concentrations of test substance in replicate test chambers should not vary more than ± 20%.	• Test substance concentrations were measured prior to test initiation and at days 0, 7, 14 and 21 during the in-life portion of the test. (pp. 24, 32) • The nominal test concentrations were 0.22, 0.44, 0.88, 1.8, and 3.5 µg ai/L (p. 16). The mean measured concentrations were determined to be 0.13, 0.20, 0.57, 1.0, and 2.0 µg ai/L. (pp. 24, 32)
Controls • Controls should consist of same dilution water, conditions, and procedures, and daphnids. • Negative and/or solvent	• A dilution water and solvent control were run under the same conditions as the five concentrations of the test substance. (pp. 15, 16)
Replicates Per Dose • Equal number of daphnids in 2 or more replicates per dose (flow-through) • One daphnid each in 10 or more replicates per dose (static-renewal).	• The test concentration and controls contained twenty *Daphnia magna* (10 organisms per replicate test vessel). (p. 18)

Number of Organisms: • Minimum of 20 daphnids per concentration (flow-through). • Minimum of 10 daphnids per concentration (static-renewal). • Test organisms randomly or impartially placed in the test chambers. • Loading should not exceed 40 daphnids per liter of test solution in static-renewal system. • Loading in flow-through test varies depending on flow rate of test solution.	• The test concentration and controls contained twenty *Daphnia magna* (10 organisms per replicate test vessel). (p. 18) • The daphnids were impartially added, two at a time to each intermediate vessel, until each vessel contained two organisms. This was repeated until each intermediate vessel contained ten organisms. The daphnids were then introduced into the replicate exposure vessels by impartially selecting one of the unlabeled intermediate vessels containing ten organisms and gently pipetting them one at time under the surface of the test solution. (p. 18)
Duration of Test • 21 days	• Daphnids were observed for 21 days. (p. 18)
Observation of Daphnids • Daphnids in the test chambers observed on day 21 of the test. • Offspring should be counted and removed from the test chambers every 2 or 3 days. • Abnormal behavior or appearance reported.	• The number of immobilized offspring and adult daphnids and observations of abnormal behavior were recorded on days 0, 2, 4, 7, 11, 13, 14, 15, 18, 19, 20 and 21. (p. 18) • Assessment of the offspring was determined on day 7 and three times per week through day 21. (p. 18)
Test Endpoints Measured • Number of daphnids immobilized (EC_{50} values and 95% C.I.) • Number of young per adult. • MATC determined for most sensitive endpoint.	• The mean measured concentrations tested and the corresponding data for immobilization and reproduction derived from the definitive toxicity test were used to estimate the 21-day EC50 and 95% CI. (p. 22) • The cumulative number of offspring per female in each vessel was determined by dividing the number of counted offspring by the number of surviving female daphinds. (p. 21) • The MATC was calculated based on the LOEC and NOEC. The determination of these levels was based on the most sensitive of the performance criteria evaluated, reproduction and growth. (p. 22)
Growth • Determined by measuring total body length or dry weight (both preferred).	• At test termination, the total body length and dry weight of each surviving adult daphnid was measured. (p. 19)

Validity of Test Test is only valid if: • Less than 20% of the control should be immobilized, stressed, or diseased at the end of the study. • Each control daphnid should have produced at least 60 young after 21 days. • The controls should not produce any ephippia.	• After 21 days of exposure, survival among the control and solvent control organisms both averaged 100%. (p. 26) • The cumulative number of offspring released by each female organism of the control and solvent control organisms was 164 and 174 offspring, respectively. (p. 25) • It was not reported whether the controls did not produce any ephippia.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes (pp. 3, 4)
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	• Yes (cover page and p. 9)
Growth of the daphnids determined by total body length or body weight?	• Yes (p. 19)
Source of test material, lot number, composition, known chemical and physical properties, and any carriers or other additives used and their concentrations reported?	• Yes (pp. 9, 13, 16)
Source of the dilution water, its chemical characteristics (e.g. conductivity, hardness, pH), and a description of any pretreatment reported?	• Yes (p. 15)
Detailed information about the daphnids provided?	• Yes (p. 14)
Description of the test chambers provided?	• Yes (pp. 15-17)
Concentration of the test substance in the test chambers at the designated times provided?	• Yes (pp. 24, 25)
Number and percentage of organisms that showed any adverse effect reported?	• Yes (p. 26)
Cumulative adult and offspring immobilization values, progeny produced, the time to first brood, the number offspring per adult, and growth of surviving adults measured?	• Yes (pp. 25-27)
All chemical analysis (of water quality) and test substance concentrations, including methods, method validation, and reagents reported?	• Yes (pp. 20, 31, 32, App. A - p. 54)

Data records of the culture, acclimation, and test temperature provided?	• Data records for the water temperature during the test were provided (p. 31). Data on temperature during culture and acclimation were not reported.
Deviations from the test guideline provided and anything unusual about test (e.g., dilution failure, temperature fluctuations) reported?	• Yes (p. 28).
MATC reported and statistical methods employed reported?	• Yes (pp. 21, 22).
Concentration-response curves utilizing average test substance concentration and adult immobilization data at 21 days provided?	• Graphs were provided. (p. 38)
EC50 value based on adult immobilization calculated using the average measured concentration of the test substance?	• Yes (p. 22)
Raw data included:	• Yes, some raw data were provided for each replicate for length and dry weight; however, only mean values per concentration level were provided for percent survival and number of offspring produced. (p. 78-81)
Statistical methods reported:	• Yes (pp. 20-22)

Dose Response

Nominal Concentration (%)	Mean Concentration (mg/L)	Survival (%)	Mean Cumulative No. Offspring Produced Over 21 Days	Mean Total Body Length at Day 21 (mm)	Mean Total Dry Weight at Day 21 (mg)
Control	<0.072	100	164	4.79	1.08
Solvent Control	<0.072	100	174	4.77	1.16
0.22	0.13	95	176	4.80	1.11
0.44	0.20	100	165	4.83	1.15
0.88	0.57	100	135	4.54	0.87
1.8	1.0	5	34	4.10[a]	0.51[a]
3.5	2.0	0	1	NA[b]	NA[b]

a based on measurements provided for one surviving organism in one replicate of the dose.
b No surviving organisms.

Statistical Results

Statistical Method: The following statistical procedures were utilized:

- Significant differences in percent survival were evaluated after transformation of the data.
- Shapiro Wilk's Test for normality was used to compare the observed sample distribution with a normal distribution for survival, reproduction and growth.
- Bartlett's Test was used as a check on the assumption of homogeneity of variance, data for survival, reproduction, length and weight.
- A t-test was used to compare the survival, reproduction and growth of the control to that of the solvent control.
- If the data passed the two tests for normality and homogeneity of variance, then a parametric method was used to evaluate the results of the life-cycle test. If the data failed the tests for either normality or homogeneity of variance, then a non-parametric method (Dunn's or Steel's One Many Rank Test) was used to evaluate the results of the life-cycle test.
- The MATC was determined based on the limits set by the LOEC and the NOEC. The determination of these levels is based on the most sensitive of the performance criteria evaluated.
- The mean-measured concentrations tested and the corresponding data for immobilizing and reproduction derived from the definitive toxicity test were used to estimate the 21-day median effect concentration (EC50) and the corresponding 95% CI. Three methods were available: moving average angle analysis, probit analysis, and binominal probability.

Results Synopsis:

NOEC = 0.20 µg ai/L
LOEC = 0.57 µg ai/L
MATC = 0.34 µg ai/L
EC50 (95% CI) = 0.78 µg ai/L (0.57 to 1.0 µg ai/L)

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: A parametric t-test was used to determine if there was a statistical difference between the control and solvent control data. If no difference was observed, the control data were pooled for further statistical analyses. Pooled control data were compared to treatment means. Data were first analyzed for normality and homogeneity of variances. If the data were not found to be normally distributed or the variances not homogenous, the Wilcoxan Rank Sum Test or the Steel's Many One Rank Test was used to compare control and treatment means and to determine the NOEC. Otherwise, Dunnett's Test was used to determine the NOEC. The EC50 was determined using the Toxanal program that provides results from three statistical methods: the binomial method, the moving average method, and the probit method.

Verified Results Synopsis:

EC50
Versar could not verify the EC50 value. The study value was based on survival and reproductive success. Although versar used mean survival data within the computer program Toxanal; the program results were inconclusive and should not be utilized. Individual organism reproductive successes were not provided in order to run EPA's Linear Interpolation program, as an alternative.

NOEC/LOEC
Based on both dry weight and length:

NOEC = 0.44 µg ai/L
LOEC = 0.88 µg ai/L

**Data for the 1.8 mg a.i./L and 3.5 mg a.i./L were not included in verification of the NOEC and LOEC. There was a statistical significance that arises due to the fact that a majority of the organisms were dead and not included in the data sets for length as well as dry weight.

MATC
Versar calculated the MATC by taking the geometric mean of the NOEC and LOEC. The MATC was determined to be 0.40 ug a.i./L and matched the result stated in the study report.

MATC = 0.62 µg ai/L

14. REVIEWER'S COMMENTS: Versar's verified NOEC and LOEC do not agree with study results. This can be attributed to a number of factors including the use of differing computer programs and statistical techniques.

EC50 Based on Survival-TOXANAL-Inconclusive

45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	29.35302	88.50114	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.816341
95 PERCENT CONFIDENCE LIMITS =-21.27784 AND 30.91052

LC50 = 1.199483
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .6535945
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
****
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
?

Length- NOEC/LOEC-TOXSTAT

Length
File: R Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	4.776	4.776		
2	0.22	4.800	4.800	-0.834	
3	0.44	4.830	4.830	-1.919	
4	0.88	4.542	4.542	8.347	*

Bonferroni t table value = 2.16 (1 Tailed Value, P=0.05, df=90,3)

Length
File: R Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

	NUM OF	Minimum Sig Diff	% of	DIFFERENCE

GROUP	IDENTIFICATION	REPS	(IN ORIG. UNITS)	CONTROL	FROM CONTROL
1	GRPS 1&2 POOLED	40			
2	0.22	19	0.062	1.3	-0.024
3	0.44	20	0.061	1.3	-0.054
4	0.88	20	0.061	1.3	0.234

Dry Weight- NOEC/LOEC

Dry Weight
File: R2 Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

		TRANSFORMED MEAN	CALCULATED IN		
ROUP	IDENTIFICATION	MEAN	ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	1.122	1.122		
2	0.22	1.115	1.115	0.220	
3	0.44	1.154	1.154	-0.909	
4	0.88	0.865	0.865	7.419	*

Bonferroni t table value = 2.16 (1 Tailed Value, P=0.05, df=90,3)

Dry Weight
File: r2 Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	40			
2	0.22	19	0.076	6.8	0.008
3	0.44	20	0.075	6.7	-0.032
4	0.88	20	0.075	6.7	0.257

DATA EVALUATION RECORD
AVIAN DIETARY TOXICITY TEST
GUIDELINE OPPTS 850.2200

1. **CHEMICAL:** ECONEA Technical **PC Code No.:**

2. **TEST MATERIAL:** R107894 **Purity:** 94.6%
Lot No. AC 12649-8

3. **CITATION**

Authors:	Sean P. Gallagher, Kathy H. Martin, Joann B. Beavers
Title:	R107984: A Dietary LC50 Study With the Mallard duck (*Anas platyrhynchos*)
Study Completion Date:	July 8, 2005
Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse Belgium
Laboratory Report ID:	Janssen Study Number: AGR 916 Wildlife International, Ltd. Project Number: 168-101B
MRID No.:	465960-05

4. **REVIEWED BY:**

Signature: Date: 4/11/06
DABCE/ABD

5. **APPROVED BY:**

Signature: Date: 4/17/06

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Anas platyrhynchos*
Age of Test Organism:	10 days at test initiation
Definitive Test Duration:	11 days (May 26, 2005 to June 6, 2005)
Study Method:	Static
Type of Concentrations:	Nominal

7. **CONCLUSIONS**

Results Synopsis:

Dietary LC_{50}: 10.76 ppm a.i.
95% Confidence Intervals: 5.62 to 17.8 ppm a.i.

No-mortality level: 5.62 ppm a.i.

Growth (based on reductions in mean body weight gain)
NOEC: 10 ppm a.i.
LOEC: 5.62 ppm a.i.

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale:

C. Repairability:

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.2200:

- The study reported that the external walls, ceilings, and floors of the pens were constructed of vinyl coated wire grid. The guidelines state that the pens should be constructed of galvanized metal, stainless steel, or perfluorocarbon plastics and that wire mesh should be used for floors and external walls.
- The relative humidity was sometimes higher than the guideline recommended range of 45-70%. The relative humidity in the study ranged from 66-78%.
- The study did not report if the diet was analyzed for contaminants or if water pans or bowls were used.
- Test concentrations for this study were selected after two previous tests of higher test concentrations produced mortality in the lowest test concentrations. These concentrations ranged from 5.62 ppm to 1780 ppm in the two previous tests. The guidelines state that a range-finding test using groups of a few birds fed 3 to 5 widely spaced concentrations for 5 days should be conducted.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information

Species ▪ Preferred species: either an upland game bird species, preferably the bobwhite (*Colinus virginianus*) or a wild waterfowl species, preferably the mallard (*Anas platyrhynchos*). ▪ If bobwhite purchased, preferable that purchased as eggs which are hatched and reared in testing facility ▪ During incubation of bobwhite quail, recommended temperature is 39°C and relative humidity is 70% ▪ All birds used in test should be from same source and hatch	▪ Mallard (*Anas platyrhynchos*). (p. 8) ▪ Yes. (p. 11)
Age at beginning of test ▪ Bobwhite quail: 10-14 days old ▪ Mallard duck: 5-10 days old ▪ All treatment and control birds should be same age ±1 day. ▪ Exact age should be reported.	▪ 10 days old. (p. 11) ▪ All mallards were 10 days of age. (p. 11) ▪ Study reports that all mallards were 10 days old. (p. 11)
Chicks appeared healthy and did not have excessive mortality before the test? ▪ Birds should not be used for test if more than 5% of total test population die during 72 hours preceding test	▪ Reports states that all mallards appeared to be in good health at test initiation. (p. 11) Pre-test mortality rates were not provided.
Acclimation period ▪ Acclimated to test facilities and diet for a minimum of 7 days	▪ Acclimated for 8 days. (p. 8, 10)

B. Test System

Guideline Criteria	Reported Information
Pens ▪ Should be constructed of galvanized metal, stainless steel, or perfluorocarbon plastics ▪ Wire mesh should be used for floors and external walls ▪ Floor area should be at least 300 cm^2/bird for bobwhite quail and 600 cm^2/bird for mallard duck ▪ Should be kept indoors and heated	▪ External walls, ceilings, and floors were contructed of vinyl coated wire grid. (p.14) ▪ Floor space was 62 x 92 cm (5704 cm^2) for each pen. 5 ducklings in each pen so area was 1140.8 cm^2/bird. (p. 14) ▪ Yes. (p. 14)
Room temperature ▪ 22-38°C	▪ Pen brooding compartments: 30.5 ± 1.2°C ▪ Ambient Room: 23.9 ± 0.6°C (p. 14)
Relative humidity ▪ 45-70%	▪ 72 ± 6%. (p. 14)

Photoperiod ▪ Recommended 14 hours light/10 hours dark ▪ Continuous lighting is acceptable	▪ 16 hours light per day. (p. 14)
Diet ▪ A commercial diet for game birds or duck starter mash should be used ▪ Only clean, unmedicated water should be offered during 96 hours preceding test period ▪ Diets should be analyzed periodically for contaminants ▪ Nutrient analysis and list of ingredients in diet should be included in report ▪ Clean water should be available ad libitum; if water pans or bowls used water should be changed at least once a day ▪ Nutrient analysis of diet should be included in report and a list of ingredients for commercially prepared diets	▪ Fed a game bird ration. (p. 11) ▪ Water was from a public water supply and birds received no form of antibiotic medication. (p. 11) ▪ Information on contaminants in diet was not provided. ▪ Yes. (Appendix II and III) ▪ Water and feed were provided ad libitum. (p. 11) Study did not report the use of pans or bowls.

C. **Test Design**

Guideline Criteria	Reported Information
Range finding test ▪ Should be conducted ▪ Generally, groups of a few birds fed 3 to 5 widely spaced concentrations for 5 days ▪ Concentration series of 5, 50, 500, and 5,000 ppm suggested	▪ Test concentrations for this study were selected after two previous tests of higher test concentrations (5.62 ppm to 1780 ppm) produced mortality in the lowest test concentrations. (p. 9)
Test Concentrations ▪ Minimum of 5 concentrations spaced geometrically ▪ Recommended spacing is for each concentration to be at least 60% of next highest dose ▪ At least one concentration should kill more than 50% and at least one concentration should kill less than 50% ▪ Treated diets should be analyzed to confirm proper dietary concentration of test substance–should be conducted at beginning of exposure period with samples from high, middle and low concentrations	▪ 0, 1.78, 3.16, 5.62, 10, 17.8, and 31.6 ppm a.i. spaced by a factor of 1.78 (p. 11) ▪ Spacing is between 50-60%. ▪ Yes. (p. 15-16) ▪ Yes. (p. 12, 15)

Controls ▪ Concurrent control group required ▪ Should be from same hatch as those used in treatments ▪ Kept under same environmental conditions	 ▪ Yes (p. 11) ▪ Yes (p. 11) ▪ Yes (p. 14)
Number of birds per group ▪ Minimum of 10 per test concentration ▪ Minimum of 20 for negative or carrier controls; 30 or more control birds is preferred	 ▪ Each treatment group assigned two pens with 5 ducklings each (10 per concentration). Control group assigned six pens with 5 duckling each (30 for the control). (p. 14)
Test Substance ▪ Should be mixed in diet evenly ▪ Should be added without use of diluent; if needed preferred diluent is distilled water or if substance is not water soluble, reagent grade evaporative diluent (e.g., acetone or methylene chloride) ▪ Other possible diluents: corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic ▪ If diluent used, should not comprise more than 2% by weight of treated diet ▪ Diets can be mixed by commercial, mechanical food mixers and may be mixed under a hood ▪ Should be mixed freshly just prior to beginning of test	 ▪ Yes. (Appendix III) ▪ The study does not mention use of any diluents. ▪ Mixed on a Hobart (Model Number AS200T) mixer. (p. 11) ▪ Diet prepared on the day of test initiation. (p. 11)
Test Acceptability ▪ No more than 10% of control birds die ▪ Evidence provided that test concentrations were at least 80% of nominal for first 5 days of test period ▪ Lowest treatment level did not result in compound-related mortality or other observable effects	 ▪ No mortalities in the control group. (p. 15, 19-20) ▪ Concentrations were at least 80% of nominal for first 5 days. (p. 32-Appendix IV) ▪ No mortalities in lowest treatment level (1.78 ppm) (p. 15, 19-20)
Test durations ▪ 5 days with treated feed and at least 3 days observation with "clean" feed ▪ If any test birds die during 2^{od} or 3rd day of postexposure period, test period should be extended until 2 successive mortality-free days and 1 day free of toxic signs occur or until 21 days after beginning of test (whichever comes first)	 ▪ Acclimation was 8 days, exposure was 5 days (Day 0-5), and post-exposure observation was 6 days (Day 6-11). (p. 13) ▪ Additional deaths did not occur in the test birds during the post-exposure period (p. 19-20.

Observations ▪ Signs of intoxication, abnormal behavior and mortality should be recorded and reported by dose level and by day ▪ Should be made at a minimum 3x on the first day of exposure ▪ Should be made at least twice during remainder of test period; twice daily observations recommended ▪ Average body weights should be reported at beginning and end of normal 3-day postexposure period ▪ Average food consumption should be measured either daily or every other day in controls and pens with second lowest and second highest concentration levels; for other pens should be measured for both the exposure period and the normal 3-day postexposure period	▪ Record was maintained of all signs of toxicity and abnormal behavior. (p. 14, 19-20, Appendix V) ▪ All birds were observed four times on the day of experimental start and at least twice daily throughout the test. (p. 14) ▪ Individual body weights were measured on Days 0 (initiation), 5, 8 and 11 (termination). (p. 14, 21) ▪ Average feed consumption values were determined daily during the exposure period (Days 0-5) and twice during the post-exposure observation period (Days 6-8 and 9-11) by pen for each treatment group and the control group. (p. 14, 22)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. (p. 3, 4)
Name of test, sponsor, test laboratory and location, principal investigators and actual dates of beginning and end of test reported?	Yes. (Title page, p. 8)
Name of test species, age, average body weights and individual body weights of all birds that die during test reported?	Yes. (p. 21, Appendix VI)
Description of housing conditions (type, size and material of pen, temperatures, humidity, photoperiod and lighting intensity) reported?	Yes. (p. 14)
Detailed description of diet (source, diluents, supplements, if used) reported? Nutrient analysis of diet included?	Yes. (p. 11, Appendix II and III)
Detailed description of test substance including chemical name, source, composition, physical/chemical properties reported?	Chemical name and source reported. (p. 10)
Number of concentrations used, nominal and measured concentrations, number of birds per concentration and for controls reported?	Yes. (p. 11)
Acclimation procedures reported?	Yes. (p. 10-14)
Frequency, duration and methods of observation reported?	Yes. (p. 14, 19-22, Appendix V)
Signs of toxicity (if any) were described?	Yes. (p. 14, 19-20, Appendix V)

Raw data included?	Yes. (Appendix V, VI, VII)

Dose Response

Mortality

Nominal Concentration (ppm a.i.)	No. of Birds	Cumulative Number of Dead											
		Day of Study											
		Exposure Period						Post Exposure Period					
		0	1	2	3	4	5	6	7	8	9	10	11
0	30	0	0	0	0	0	0	0	0	0	0	0	0
1.78	10	0	0	0	0	0	0	0	0	0	0	0	0
3.16	10	0	0	0	0	0	0	0	0	0	0	0	0
5.62	10	0	0	0	0	0	0	0	0	0	0	0	0
10	10	0	0	1	2	3	4	4	4	4	4	4	4
17.8	10	0	0	2	6	9	10	10	10	10	10	10	10
31.6	10	0	0	6	9	10	10	10	10	10	10	10	10

Mean Body Weights

Nominal Concentration (ppm a.i.)	Mean Body Weights (SD) (g)							
	Day of Study							
	Exposure Period			Post Exposure Period				Total Change*
	0	Change*	5	Change*	8	Change*	11	
0	139 (22)	142 (20)	281 (39)	98 (21)	379 (55)	100 (15)	479 (51)	340 (35)
1.78	144 (27)	139 (14)	283 (38)	100 (21)	383 (55)	117 (14)	500 (51)	356 (33)
3.16	141 (22)	128 (22)	269 (42)	116 (13)	385 (50)	111 (16)	496 (43)	355 (30)
5.62	144 (22)	108 (13)	252 (24)	105 (15)	356 (30)	105 (8)	461 (31)	317 (19)
10	147 (19)	29 (26)	175 (42)	108 (33)	283 (74)	94 (3)	376 (73)	230 (58)
17.8	-	-	-	-	-	-	-	-

31.6	-	-	-	-	-	-	-	-

*Study Report stated that mean change was calculated separately from the mean body weights using individual body weights provided in the Appendix to the report.
-No data available due to mortality

Statistical Results

Statistical Method: Mortality data was analyzed using the computer program of C.E. Stephan. For this study, the program calculated the LC_{50} value and 95% confidence interval by nonlinear interpolation. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight. The NOEC was based upon reductions in mean body weight gain during the exposure period at the 3.16 ppm a.i. test concentration.

Results Synopsis:

Survival
LC_{50}: 10.8 ppm a.i.
95% Confidence Intervals: 10 to 17.8 ppm a.i.

No-mortality level: 5.62 ppm a.i.

Growth (based on reductions in mean body weight gain)
NOEC: 1.78 ppm a.i.

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: The LC_{50} value for the mortality data was calculated with the TOXANAL program. The reductions in mean body weight gain were analyzed to determine if there were any statistically significant treatment effects. The weight data were first checked for normality using the Shapiro-Wilks test and for homogeneity of variances using Bartlett's test. The length data passed for both normality and homgogeneity of variance. The NOECs and LOECs were then determined using Bonferroni's T-test. There was no mortality observed in the control to the 5.62 ppm a.i. test concentration.

Results Verification Synopsis:

Survival
LC_{50}: 10.76 ppm a.i.
95% Confidence Intervals: 5.62 to 17.8 ppm a.i.

No-mortality level: 5.62 ppm a.i.

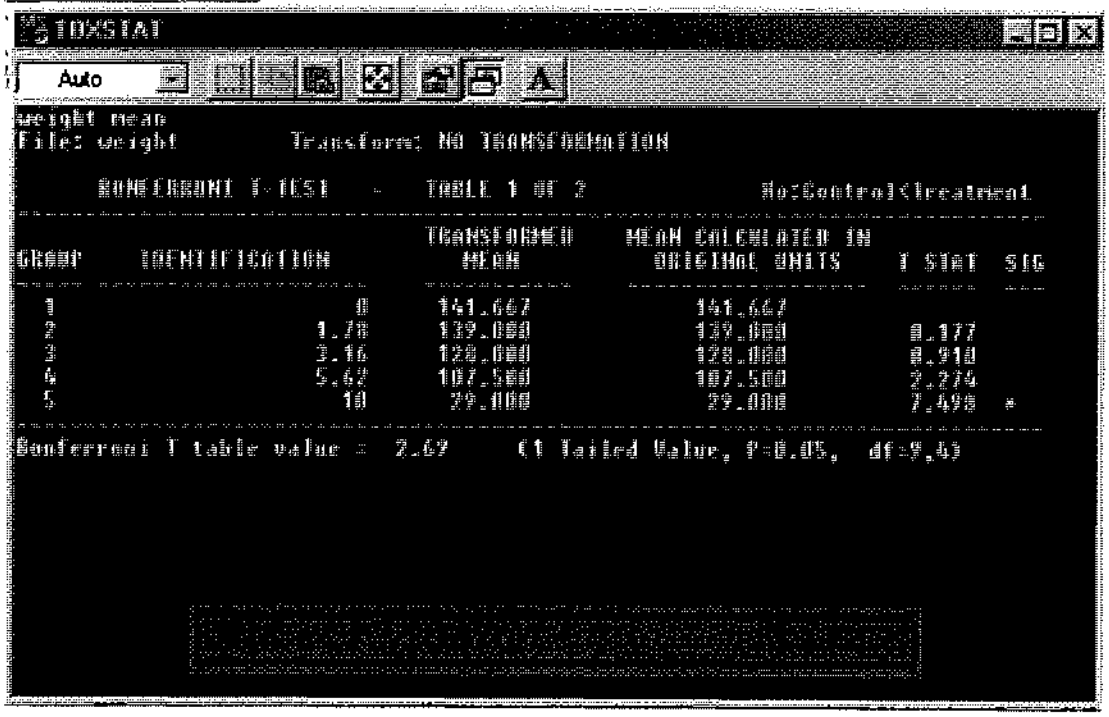
Growth (based on reductions in mean body weight gain)
NOEC: 10 ppm a.i.
LOEC: 5.62 ppm a.i.

14. REVIEWER'S COMMENTS:

- Guideline deviations are provided in Section 9.
- The lower limit for the 95% confidence levels for the LC_{50} value was lower than that reported by the study author.
- The NOEC value calculated for the weight data were different than that reported by the study author. This difference may be due to the use of different statistical tests.

LC_{50} Determination:
See attached results page.

Growth NOEC/LOEC



DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** ECONEA Technical **PC Code No.:** 119093

2. **TEST MATERIAL:** R10894 **Purity:** 94.6%

3. **CITATION:**

Author:	Hoberg, James R.
Title:	R107894—Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Under Static Conditions
Study Completion Date:	March 17, 2005
Laboratory:	Springborn Smithers Laboratories, 790 Main St. Wareham MA 02571-1075
Sponsor:	Janssen Pharmaceutica NV, Plant and Material Protection Division, Turnhoutseweg 30, B-2340 Beerse, Belgium
Laboratory Report ID:	13751.6147
DP Barcode:	
MRID No.	465960-06

4. **REVIEWED BY:**

Signature: **Date:** 1/19/06
David C. Bays, RASSB, AD (7501C)

5. **APPROVED BY:**

Signature: **Date:** 1/19/06
Rick Petrie, Team 3 Leader, RASSB, AD HCook for

Kathryn Montague, Acting Team 1 Leader, RASSB, AD HCook for

6. **STUDY PARAMETERS**

Definitive Test Duration: 96 hours
Type of Concentrations: Nominal

7. **CONCLUSIONS**

Results Synopsis: A significant reduction in cell density was detected in the 0.0096 and 0.014 mg a.i./L treatment levels. Based on the Williams' Test, the 96-hour NOEC was determined to be 0.0068 mg a.i./L. The 96-hour EC50 value was determined to be 0.011 mg a.i./L, with 95% confidence intervals of 0.0105 to 0.011 mg a.i./L.

Verified Results Synopsis: No calculation errors were found in the review of statistical calculations. The Dunnet's test showed statistically significant differences in the same dose groups as the study author's Williams' test.

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: The starting number of algal cells was too low (1,000 instead of 10,000)

C. Repairability: The study is not repairable because the number of starting algal cells cannot be changed and this is a major protocol deviation.

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.5400:

- The following items were not reported in the study report:
  - Sterilization/cleaning practices
  - Water solubility
  - Physical/chemical properties of the chemical, including saturation concentration
  - ~~The maximum labeled rate~~ MCook
- The lowest concentration of the range-finding test (0.0010 mg a.i./L) was not at the detection limit (0.000011 mg a.i./L).
- ~~Only two replicates per dose/control group were used in the range-finding test, instead of three.~~ MCook
- ~~No positive control was used.~~ MCook

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species •*Selenastrum capricornutum (Raphidocelis subcapitata)* •*Skeletonema costatum* •*Anabaena flos-aquae* •*Navicula pelliculosa*	*Pseudokirchneriella subcapitata* was used.
Initial Number of Cells •10,000 cells/mL (*Selenastrum, Anabaena, Navicula)* •77,000 cells/mL (*Skeletonema*)	Approximately 1,000 cells/mL. p16

Stock Culture •3 to 7 days old	Four days. p14
Nutrients •Standard formula (ASTM E1218-20) •pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) •Freshly prepared	• Sterile medium used • pH=7.5± 0.1

B. Test System

Guideline Criteria	Reported Information
Solvent Upper limit - 0.5 mL/L	• 0.1 mL/L. p15
Temperature •24° ± 2°C (*Selenastrum, Navicula, Anabaena*) •20° ± 2°C (*Skeletonema*) •Recorded hourly	• 24°C±2°C. p24,30 • Temperature recorded continuously. p17
Light Intensity •4.3 K lx (±10%) (*Selenastrum, Skeletonema, Navicula*) •2.2 K lx (±10%) (*Anabaena*) •Photosynthetically active radiation approx. 66.5 ± 10% μEin/m^2/sec	• 3.9 to 4.7 K lx. p30
Photoperiod •14-hr light/10-hr dark (*Skeletonema*) •Continuous (*Selenastrum, Navicula, Anabaena*)	Continuous. p17
pH •pH of nutrient medium: 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*) 8.1 ± 0.1 (*Skeletonema*) •Measured at beginning and end of test	• Nutrient medium pH = 7.5±0.1 p14 • Measured at beginning and end of test. p30
Oscillation Rates •100 cycles/min (*Selenastrum*) •60 cycles/min (*Skeletonema*)	• 10±10 rpm. p17
Test Containers •125-500 mL Erlenmeyer flasks •Cleaned/sterilized (solvent and acid) and conditioned •Test solution volume ≤ 50% of flask volume	• 250 mL Erlenmeyer flasks p15 • Conditioned, but sterilization/cleaning not reported • Test solution volume = 100 mL. p15
Dilution Water •Sufficient quality (e.g., ASTM Type I) •Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	• Artificially enriched seawater used (salinity = 30±2 g/L). p13

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test •Water solubility and physical-chemical properties of test chemical determined? •Validated analytical method developed? •Expose algae to widely spaced (e.g. log interval) chemical concentration series •Lowest value should be at detection limit •Upper value, for water soluble compounds, should be at saturation concentration •Minimum of 3 replicates •Algae should be exposed for 96 hours •If highest concentration (saturation concentration or 100 mg/L) results in <50% reduction in growth, definitive test may not be necessary •If lowest concentration (detection limit) results in >50% reduction, definitive test necessary	• Water solubility, physical/chemical properties could not be found in the study report. p19 • Validated method. p48 • Log intervals used. p19 • Lowest concentration of range-finding test (0.0010 mg a.i./L) p19 not at detection limit (0.000011 mg a.i./L). p58 Saturation concentration not reported. • Two replicates per dose/control group p19 • 96 hours of exposure • Definitive test justified based on results from range finding test
Dose Range •1.5X -2X progression	• 2.0X progression calculated from doses
Doses •5 or more concentrations of test substance in a geometric series •> 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	• 5 doses in a geometric series • 97% inhibition at highest doses. p33
Controls •Negative and/or solvent each test •Positive - zinc chloride (periodically)	• Negative and solvent controls used • No positive control
Replicates Per Dose •3 or more (4 or more for *Navicula*)	• Three replicates/dose. p15
Duration of Test •96-hr	• 96 hour duration.
Growth •Logarithmic growth (controls) by 96-hr or repeat test (increase by a factor of 16) •1.5 x 10^6 cells/mL (*Skeletonema*) •3.5 x 10^6 cells/mL (*Selenastrum*)	• 1.67x10^6 cell/mL at 96 hrs (average of control and solvent control). However, the study author states that log phase growth was occurring by the 96-hr observation interval. p33,26

•Daily Observations?	Yes p16
Method of Observations •Direct - microscopic cell count of at least 400 cells/flask •Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count •Qualitative and descriptive	Direct method used. p15 At least 400 cells counted. p16
Cell Separation •Syringe ultrasonic bath, or blender; limited sonification (*Anabaena*) •Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula)*	No report of filament-breaking could be found in the study report.
•Algistatic and algicidal effects differentiated?	Yes. Algistatic effect determined. p16
•Maximum Labeled Rate	It is unclear if the maximum labeled rate was used.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes.
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	Yes. p13
Growth in controls reported?	Yes. p30
Description of test system and test design included?	Yes.
Initial and final chemical concentrations and pH measured?	Yes.
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	Yes.
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	Yes.
Raw data included?	Yes. p30
Methods and data records reported?	Yes. p18, appendix 2
Statistical Analysis •Mean and standard deviation calculated and plotted? •Goodness-of-fit determined?	Yes.

Dose Response

Nominal Concentration (mg a.i./L)	Initial Measured Concentration (mg a.i./L)	Final Measured Concentration (mg a.i./L)	Cell Density at 96 hrs ($x 10^4$ cells/mL)	% Inhibition (reduction in growth rate compared with pooled control/ solvent control data)	pH	
					0-hr	96-hr
Control	<0.00085	<0.00088	151.89±19.46	NA	7.3	9.3
Solvent Control	<0.00085	<0.00088	183.83±32.35	NA	7.2	8.9
Pooled Control			167.86±29.60	NA	--	--
0.0063	<0.00085	0.0037	146.67±9.68	13	7.4	9.3
0.013	0.0018	0.0074	173.72±28.36	-3	7.3	9.2
0.025	<0.0042	0.015	155.44±29.36	7	7.4	9.4
0.05	0.0055	0.032	120.08±4.71	28	7.4	9.0
0.1	0.010	0.068	5.83±1.76	97	7.3	7.5

Statistical Results

Statistical Method: A t-test was used to compare the daily cell density of the control to the solvent control. The solvent control was used for comparison to treatment data if a significant difference was determined; otherwise, the control and solvent control data were pooled and used for comparison. EC50 values were calculated using TOXSTAT. The NOEC was determined by determining the highest test concentration which demonstrated no statistically adverse effect (p 0.05). Normality was checked using Shapiro-Wilks' Test, and homogeneity of variance was checked using Bartlett's Test. If the data sets passed the test for homogeneity and normality, then Williams' Test was used to determine the NOEC.p18

Results Synopsis: Because no significant difference was determined between the control and solvent control data, the pooled control and solvent control data were used for comparison to treatment data. The cell density data were found to be normally distributed and have homogeneity of variance; therefore, the Williams' Test was used to determine treatment-related effects. A significant reduction in cell density was detected in the 0.0096 and 0.014 mg a.i./L treatment levels. Based on the Williams' Test, the 96-hour NOEC was determined to be 0.0068 mg a.i./L. The 96-hour EC50 value was determined to be 0.011 mg a.i./L, with 95% confidence intervals of 0.0105 to 0.011 mg a.i./L.

13. **VERIFICATION OF STATISTICAL RESULTS**

Statistical Method: Calculations of cell density averages and standard deviations were checked by Versar for

accuracy. EC50 calculations were inspected for reasonableness with respect to the raw data. In order to verify calculations of the 96-hr NOEC, the Dunnet's test ($p<0.05$) was performed on the cell density data.
Results Verification Synopsis: No calculation errors were found in the review of statistical calculations. The Dunnet's test showed statistically significant differences in the same dose groups as the study author's Williams' test.

14. REVIEWER'S COMMENTS:

The following guideline deviations were found in the study report:

- The following items were not reported in the study report:
  - Sterilization/cleaning practices
  - Water solubility
  - Physical/chemical properties of the chemical, including saturation concentration
  - ~~The maximum labeled rate~~ NCWK
- The lowest concentration of the range-finding test (0.0010 mg a.i./L) was not at the detection limit (0.000011 mg a.i./L).
- ~~Only two replicates per dose/control group were used in the range-finding test, instead of three.~~ NCWK
- ~~No positive control was used.~~ NCWK

DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** ECONEA Technical **PC Code No.:** 119093

2. **TEST MATERIAL:** R10894 **Purity:** 94.6%

3. **CITATION:**

Author:	Hoberg, James R.
Title:	R107894—Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*, Under Static Conditions
Study Completion Date:	March 17, 2005
Laboratory:	Springborn Smithers Laboratories, 790 Main St. Wareham MA 02571-1075
Sponsor:	Janssen Pharmaceutica NV, Plant and Material Protection Division, Turnhoutseweg 30, B-2340 Beerse, Belgium
Laboratory Report ID:	13751.6147
DP Barcode:	
MRID No.	465960-06

4. **REVIEWED BY:**

Signature: **Date: 10/12/06**
David C. Bays, RASSB, AD (7501C)

5. **APPROVED BY:**

Signature: **Date: 10/12/06**
Rick Petrie, Team 3 Leader, RASSB, AD

Kathryn Montague, Acting Team 1 Leader, RASSB, AD

6. **STUDY PARAMETERS**

Definitive Test Duration: 96 hours
Type of Concentrations: Nominal

7. **CONCLUSIONS**

Results Synopsis: A significant reduction in cell density was detected in the 0.0096 and 0.014 mg a.i./L treatment levels. Based on the Williams' Test, the 96-hour NOEC was determined to be 0.0068 mg a.i./L. The 96-hour EC50 value was determined to be 0.011 mg a.i./L, with 95% confidence intervals of 0.0105 to 0.011 mg a.i./L.

Verified Results Synopsis: No calculation errors were found in the review of statistical calculations. The Dunnet's test showed statistically significant differences in the same dose groups as the study author's Williams'

test.

8. ADEQUACY OF THE STUDY

A. **Classification: Core**

B. **Rationale: Scientifically acceptable study**

C. **Repairability: The study was upgraded after receiving information (MRID 469179-01) resolving the guideline deviations. (See below)**

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.5400:

- Data were provided by the registrant (MRID 469179-01) to clarify the following deviations:
  - Sterilization/cleaning practices (Pgs 13, 14, 15 describe sterilization practices)
  - Water solubility (provided in MRID's – 456730-06 and 456739-07)
  - Physical/chemical properties of the chemical, including saturation concentration (Provided in MRID's – 456730-06 and 456730-07)
  - (Sterilization/cleaning practices were reported on pages 13, 14 and 15. Water solubility and physical/chemical properties was provided by the Study Sponsor. See MRID #s 45673906, 45673907 and 46545101)
- The lowest concentration of the range-finding test (0.0010 mg a.i./L) was not at the detection limit (0.000011 mg a.i./L). (Since all effects in the study are greater that the established limit of quantition, this is not necessary)

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. **Test Organisms**

Guideline Criteria	Reported Information
Species •*Selenastrum capricornutum (Raphidocelis subcapitata)* •*Skeletonema costatum* •*Anabaena flos-aquae* •*Navicula pelliculosa*	*Pseudokirchneriella subcapitata* was used.
Initial Number of Cells •10,000 cells/mL (*Selenastrum, Anabaena, Navicula)* •77,000 cells/mL (*Skeletonema*)	Approximately 1,000 cells/mL, p16

Stock Culture •3 to 7 days old	Four days. p14
Nutrients •Standard formula (ASTM E1218-20) •pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) •Freshly prepared	• Sterile medium used • pH=7.5± 0.1

B. Test System

Guideline Criteria	Reported Information
Solvent Upper limit - 0.5 mL/L	• 0.1 mL/L. p15
Temperature •24E ± 2EC (*Selenastrum, Navicula, Anabaena*) •20E ± 2EC (*Skeletonema*) •Recorded hourly	• 24EC±2EC. p24,30 • Temperature recorded continuously. p17
Light Intensity •4.3 K lx (±10%) (*Selenastrum, Skeletonema, Navicula*) •2.2 K lx (±10%) (*Anabaena*) •Photosynthetically active radiation approx. 66.5 ± 10% ΦEin/m^2/sec	• 3.9 to 4.7 K lx. p30
Photoperiod •14-hr light/10-hr dark (*Skeletonema*) •Continuous (*Selenastrum, Navicula, Anabaena*)	Continuous. p17
pH •pH of nutrient medium: 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*) 8.1 ± 0.1 (*Skeletonema*) •Measured at beginning and end of test	• Nutrient medium pH = 7.5±0.1 p14 • Measured at beginning and end of test. p30
Oscillation Rates •100 cycles/min (*Selenastrum*) •60 cycles/min (*Skeletonema*)	• 10±10 rpm. p17
Test Containers •125-500 mL Erlenmeyer flasks •Cleaned/sterilized (solvent and acid) and conditioned •Test solution volume # 50% of flask volume	• 250 mL Erlenmeyer flasks p15 • Test solution volume = 100 mL. p15
Dilution Water •Sufficient quality (e.g., ASTM Type I) •Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	• Artificially enriched seawater used (salinity = 30±2 g/L). p13

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test •Water solubility and physical-chemical properties of test chemical determined? •Validated analytical method developed? •Expose algae to widely spaced (e.g. log interval) chemical concentration series •Lowest value should be at detection limit •Upper value, for water soluble compounds, should be at saturation concentration •Minimum of 3 replicates •Algae should be exposed for **96** hours •If highest concentration (saturation concentration or 100 mg/L) results in <50% reduction in growth, definitive test may not be necessary •If lowest concentration (detection limit) results in >50% reduction, definitive test necessary	• Water solubility, physical/chemical properties could not be found in the study report. p19 • Validated method. p48 • Log intervals used. p19 • Lowest concentration of range-finding test (0.0010 mg a.i./L) p19 not at detection limit (0.000011 mg a.i./L). p58 Saturation concentration not reported. • Two replicates per dose/control group p19 • 96 hours of exposure • Definitive test justified based on results from range finding test
Dose Range •1.5X -2X progression	• 2.0X progression calculated from doses
Doses •5 or more concentrations of test substance in a geometric series •> 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	• 5 doses in a geometric series • 97% inhibition at highest doses. p33
Controls •Negative and/or solvent each test •Positive - zinc chloride (periodically)	• Negative and solvent controls used • No positive control
Replicates Per Dose •3 or more (4 or more for *Naviculu*)	• Three replicates/dose. p15
Duration of Test •96-hr	• 96 hour duration.
Growth •Logarithmic growth (controls) by 96-hr or repeat test (increase by a factor of 16) •1.5 x 10^6 cells/mL (*Skeletonema*) •3.5 x 10^6 cells/mL (*Selenastrum*)	• 1.67x10^6 cell/mL at 96 hrs (average of control and solvent control). However, the study author states that log phase growth was occurring by the 96-hr observation interval. p33,26
•Daily Observations?	Yes p16
Method of Observations •Direct - microscopic cell count of at least 400 cells/flask •Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count •Qualitative and descriptive	Direct method used. p15 At least 400 cells counted. p16

Cell Separation •Syringe ultrasonic bath, or blender; limited sonification (*Anabaena*) •Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula*)	No report of filament-breaking could be found in the study report.
•Algistatic and algicidal effects differentiated?	Yes. Algistatic effect determined. p16

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes.
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	Yes. p13
Growth in controls reported?	Yes. p30
Description of test system and test design included?	Yes.
Initial and final chemical concentrations and pH measured?	Yes.
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	Yes.
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	Yes.
Raw data included?	Yes. p30
Methods and data records reported?	Yes. p18, appendix 2
Statistical Analysis •Mean and standard deviation calculated and plotted? •Goodness-of-fit determined?	Yes.

Dose Response

Nominal Concentration (mg a.i./L)	Initial Measured Concentration (mg a.i./L)	Final Measured Concentration (mg a.i./L)	Cell Density at 96 hrs ($x 10^4$ cells/mL)	% Inhibition (reduction in growth rate compared with pooled control/ solvent control data)	pH	
					0-hr	96-hr
Control	<0.00085	<0.00088	151.89±19.46	NA	7.3	9.3
Solvent Control	<0.00085	<0.00088	183.83±32.35	NA	7.2	8.9
Pooled Control			167.86±29.60	NA	--	--
0.0063	<0.00085	0.0037	146.67±9.68	13	7.4	9.3
0.013	0.0018	0.0074	173.72±28.36	-3	7.3	9.2
0.025	<0.0042	0.015	155.44±29.36	7	7.4	9.4
0.05	0.0055	0.032	120.08±4.71	28	7.4	9.0
0.1	0.010	0.068	5.83±1.76	97	7.3	7.5

Statistical Results

Statistical Method: A t-test was used to compare the daily cell density of the control to the solvent control. The solvent control was used for comparison to treatment data if a significant difference was determined; otherwise, the control and solvent control data were pooled and used for comparison. EC50 values were calculated using TOXSTAT. The NOEC was determined by determining the highest test concentration which demonstrated no statistically adverse effect ($p \leq 0.05$). Normality was checked using Shapiro-Wilks' Test, and homogeneity of variance was checked using Bartlett's Test. If the data sets passed the test for homogeneity and normality, then Williams' Test was used to determine the NOEC.p18

Results Synopsis: Because no significant difference was determined between the control and solvent control data, the pooled control and solvent control data were used for comparison to treatment data. The cell density data were found to be normally distributed and have homogeneity of variance; therefore, the Williams' Test was used to determine treatment-related effects. A significant reduction in cell density was detected in the 0.0096 and 0.014 mg a.i./L treatment levels. Based on the Williams' Test, the 96-hour NOEC was determined to be 0.0068 mg a.i./L. The 96-hour EC50 value was determined to be 0.011 mg a.i./L, with 95% confidence intervals of 0.0105 to 0.011 mg a.i./L.

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Calculations of cell density averages and standard deviations were checked by Versar for

accuracy. EC50 calculations were inspected for reasonableness with respect to the raw data. In order to verify calculations of the 96-hr NOEC, the Dunnet's test ($p<0.05$) was performed on the cell density data.
Results Verification Synopsis: No calculation errors were found in the review of statistical calculations. The Dunnet's test showed statistically significant differences in the same dose groups as the study author's Williams' test.

14. REVIEWER'S COMMENTS:

The following guideline deviations were addressed by the registrant in MRID 469179-01:

Sterilization/cleaning practices
Water solubility
Physical/chemical properties of the chemical, including saturation concentration
The lowest concentration of the range-finding test (0.0010 mg a.i./L) was not at the detection limit (0.000011 mg a.i./L).

DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** CL322,250 **PC Code No.:** 119093

2. **TEST MATERIAL:** CL322,250 **Purity:** 92.6%

3. **CITATION**

Author:	Hoberg, James R.
Title:	CL322,250—Acute Toxicity to the Marine Diatom, *Skeletonema costatum*, Under Static Conditions
Study Completion Date:	March 17, 2005
Laboratory:	Springborn Smithers Laboratories, 790 Main St. Wareham MA 02571-1075
Sponsor:	Janssen Pharmaceutica NV, Plant and Material Protection Division, Turnhoutseweg 30, B-2340 Beerse, Belgium
Laboratory Report ID:	13751.6147
DP Barcode:	3214543
MRID No.:	465960-14

4. **REVIEWED BY:**

Signature: **Date: 10/12/06**

David C. Bays, RASSB, AD (7510C)

5. **APPROVED BY:**

Signature:

Rick Petrie, Team 3 Leader, RASSB, AD (7510C) **Date: 10/12/06**

Kathryn Montague, Acting Team 1 Leader, RASSB, AD (7510C)

6. **STUDY PARAMETERS**

Definitive Test Duration: 96-hour
Type of Concentrations: Nominal

7. **CONCLUSIONS**

Results Synopsis: A significant reduction in cell density was detected in treatment levels ≥0.13 mg a.i./L. Because the Williams' test did not determine a NOEC, Bonferroni's Test was used. Bonferroni's Test determined a significant reduction in cell density in the 0.13 and 1.0 mg a.i./L treatment levels. However,the next two higher

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species •*Selenastrum capricornutum (Raphidocelis subcapitata)* •*Skeletonema costatum* •*Anabaena flos-aquae* •*Navicula pelliculosa*	*Skeletonema costatum* was used.
Initial Number of Cells •10,000 cells/mL (*Selenastrum, Anabaena, Navicula)* •77,000 cells/mL (*Skeletonema*)	Approximately 77,000 cells/mL. p15
Stock Culture •3 to 7 days old	Three days. p13
Nutrients •Standard formula (ASTM E1218-20) •pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) •Freshly prepared	• Sterile medium used • pH=8.1 ± 0.1

B. Test System

Guideline Criteria	Reported Information
Solvent Upper limit - 0.5 mL/L	• 0.1 mL/. Lp15
Temperature •24E ± 2EC (*Selenastrum, Navicula, Anabaena*) •20E ± 2EC (*Skeletonema*) •Recorded hourly	• 20EC±2EC. p23,27 • Temperature recorded continuously. p16
Light Intensity •4.3 K lx (±10%) (*Selenastrum, Skeletonema, Navicula*) •2.2 K lx (±10%) (*Anabaena*) •Photosynthetically active radiation approx. 66.5 ± 10% ΦEin/m²/sec	• 3.9 to 4.7 K lx
Photoperiod •14-hr light/10-hr dark (*Skeletonema*) •Continuous (*Selenastrum, Navicula, Anabaena*)	14-hr light/10-hr dark used. p16

Guideline Criteria	Reported Information
pH •pH of nutrient medium: 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*) 8.1 ± 0.1 (*Skeletonema*) •Measured at beginning and end of test	• Nutrient medium pH = 8.1±0.1. p13 • Measured at beginning and end of test. p27
Oscillation Rates •100 cycles/min (*Selenastrum*) •60 cycles/min (*Skeletonema*)	• 60±10 rpm. p13
Test Containers •125-500 mL Erlenmeyer flasks •Cleaned/sterilized (solvent and acid) and conditioned •Test solution volume # 50% of flask volume	• 250 mL Erlenmeyer flasks. p15 • Conditioned, but sterilization/cleaning not reported • Test solution volume = 100 mL. p15
Dilution Water •Sufficient quality (e.g., ASTM Type I) •Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	• Artificially enriched seawater used (salinity = 30±2 g/L). p13

C. **Test Design**

Guideline Criteria	Reported Information
Range-Finding Test •Water solubility and physical-chemical properties of test chemical determined? •Validated analytical method developed? •Expose algae to widely spaced (e.g. log interval) chemical concentration series •Lowest value should be at detection limit •Upper value, for water soluble compounds, should be at saturation concentration •Minimum of 3 replicates •Algae should be exposed for 96 hours •If highest concentration (saturation concentration or 100 mg/L) results in <50% reduction in growth, definitive test may not be necessary •If lowest concentration (detection limit) results in >50% reduction, definitive test necessary	• Water solubility, physical/chemical properties could not be found in the study report. p19 (See MRIDs – 456739-06 and 456739-07) • Validated method. p48 • Log intervals used. p19 • Lowest concentration of range-finding test (0.0010 mg a.i./L).p19; below detection limit (0.0125 mg a.i./L).p54 Saturation concentration not reported. • Two replicates per dose/control group. p19 • 96 hours of exposure • Definitive test justified based on results from range finding test
Dose Range •1.5X -2X progression	• 2.0X progression calculated from doses

Doses •5 or more concentrations of test substance in a geometric series •> 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	• 5 doses in a geometric series • 100% inhibition at highest doses. p27
Controls •Negative and/or solvent each test •Positive - zinc chloride (periodically)	• Negative and solvent controls used • No positive control
Replicates Per Dose •3 or more (4 or more for *Navicula*)	• Three replicates/dose. p15
Duration of Test •96-hr	• 96 hour duration.
Growth •Logarithmic growth (controls) by 96-hr or repeat test (increase by a factor of 16) •1.5 x 10^6 cells/mL (*Skeletonema*) •3.5 x 10^6 cells/mL (*Selenastrum*)	• Increase by more than a factor of 16. 1.49x10^6 cell/mL at 96 hrs. p30
•Daily Observations?	Yes. p16
Method of Observations •Direct - microscopic cell count of at least 400 cells/flask •Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count •Qualitative and descriptive	Direct method used. p15 At least 400 cells counted. p16
Cell Separation •Syringe ultrasonic bath, or blender; limited sonification (*Anabaena*) •Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula*)	No report of filament-breaking could be found in the study report.
•Algistatic and algicidal effects differentiated?	Yes. p16

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	Yes. p13
Growth in controls reported?	Yes. p30
Description of test system and test design included?	Yes
Initial and final chemical concentrations and pH measured?	Yes

Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	Yes
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	Yes
Raw data included?	Yes. p30
Methods and data records reported?	Yes. p18, appendix 2
Statistical Analysis •Mean and standard deviation calculated and plotted? •Goodness-of-fit determined?	Yes.

Dose Response

Nominal Concentration (mg/L)	Initial Measured Concentration (mg/L)	Final Measured Concentration (mg/L)	Cell Density at 96 hrs (x 10^4 cells/mL)	% Inhibition (reduction in growth rate compared with Pooled Control / Solvent Control)	pH	
					0-hr	96-hr
Control	<0.014	<0.015	145.58±26.07	NA	8.0	9.0
Solvent Control	<0.014	<0.015	149.67±19.91	NA	8.0	8.9
Pooled Control	NA	NA	147.63±20.86	NA	NA	NA
0.063	<0.014	<0.015	124.75±27.67	15	8.0	8.9
0.13	0.13	0.13	99.25±36.93	33	8.0	9.0
0.25	0.22	0.25	113.75±24.54	23	8.0	8.9
0.50	0.51	0.48	115.50±11.91	22	8.0	8.9
1.0	1.1	1.0	0.50±0.50	100	8.0	8.0

a The 0.063 mg/L dose group was excluded from statistical analysis because there were indications that the test solution was not fortified at the desired concentration

Statistical Results

Statistical Method: A t-test was used to compare the daily cell density of the control to the solvent control. The solvent control was used for comparison to treatment data if a significant difference was determined; otherwise, the control and solvent control data were pooled and used for comparison. EC50 values were calculated using TOXSTAT. The NOEC was determined by determining the highest test concentration which demonstrated no

statistically adverse effect ($p \leq 0.05$). Normality was checked using Shapiro-Wilks' Test, and homogeneity of variance was checked using Bartlett's Test. If the data sets passed the test for homogeneity and normality, then Williams' Test was used to determine the NOEC. p18

Results Synopsis: Because no significant difference was determined between the control and solvent control data, the pooled control and solvent control data were used for comparison to treatment data. The cell density data were found to be normally distributed and have homogeneity of variance; therefore, the Williams' Test was used to determine treatment-related effects. A significant reduction in cell density was detected in treatment levels ≥0.13 mg a.i./L. Because the Williams' test did not determine a NOEC, Bonferroni's Test was used. Bonferroni's Test determined a significant reduction in cell density in the 0.13 and 1.0 mg a.i./L treatment levels. However,the next two higher treatment levels (0.23 and 0.50 mg a.i./L) were not affected and the reduction in cell density was not considered treatment-related. Based on Bonferroni's Test the NOEC was determined to be 0.50 mg a.i./L. The 96-hr EC50 value was determined to be 0.66 mg a.i./L, with 95% confidence intervals of 0.60 to 0.70 mg a.i./L.

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Calculations of cell density averages and standard deviations were checked by Versar for accuracy. EC50 calculations were inspected for reasonableness with respect to the raw data. In order to verify calculations of the 96-hr NOEC, the Dunnet's test and Bonferroni's test ($p<0.05$) was performed on the cell density data. Data from the 0.063 mg a.i/L dose group were excluded from analysis, to be consistent with the study report.
Results Verification Synopsis: The results of the verification calculations using Dunnet's test and Bonferroni's test showed statistically significant differences in the 1.0 mg/L dose group only. This differs from the results obtained by the study author using Williams' test (statistically significant differences at all analyzed treatment levels) and Bonferroni's test (statistically significant differences in the 0.13 and 1.0 mg/L dose groups). It is unclear, without more information regarding the study author's calculations, why this discrepancy exists. No other calculation errors were found in the review of statistical calculations.

14. REVIEWER'S COMMENTS:

No additional comments.

treatment levels (0.23 and 0.50 mg a.i./L) were not affected and the reduction in cell density was not considered treatment-related. Based on Bonferroni's Test the NOEC was determined to be 0.50 mg a.i./L. The 96-hr EC50 value was determined to be 0.66 mg a.i./L, with 95% confidence intervals of 0.60 to 0.70 mg a.i./L.

Verified Results Synopsis: The results of the verification calculations using Dunnet's test and Bonferroni's test showed statistically significant differences in the 1.0 mg/L dose group only. This differs from the results obtained by the study author using Williams' test (statistically significant differences at all analyzed treatment levels) and Bonferroni's test (statistically significant differences in the 0.13 and 1.0 mg/L dose groups).

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Scientifically Acceptable Study

C. Repairability: This study was upgraded to core after receipt of missing data. (MRID 469179-01)

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.5400:

- The light intensity fell outside the range of 4.3 k Lx ± 10% on days 2 and 3, when the light intensity at three vessels was measured to be 450 to 460 footcandles (4.9 to 5.0 K lx). **(Light intensity was at the 3.9 to 4.7 K lx level for all readings)**
- The following items were not reported in the study report:
  - Sterilization/cleaning practices – **pgs. 12, 13, 14**
  - Water solubility – **MRID's 456739-06, 456739-07**
  - Physical/chemical properties of the chemical, including saturation concentration **(Sterilization/cleaning practices were reported on pages 13, 14, and 15. Water solubility and physical/chemical properties were provided by the Study Sponsor. See MRID's 456730-06, 456739-07 and 46545101)**
- Doses selected for the main test progressed by factors of 2.5-2.6 times, rather than 1.5-2 times. **(Concentrations tested were 0.063, 0.13, 0.25 0.50 and 1.0 mg ai/L. Thus a 50% dilution factor was applied and doses progressed by a factor of 2 times)**
- Although five treatment levels were created, the 0.063 mg/L data was excluded from statistical analysis because there were indications that the test solution was not fortified at the desired concentration. **(The analytical results for this treatment level were less than the limit of detection, indicating that the test solution was inadvertently not fortified at all. Since the test solution was not fortified, it was excluded for the calculation of EC values and determination of the NOEC)**

10. SUBMISSION PURPOSE: Registration

DP Barcode: 321452 MRID No: 465960-08

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE ACUTE TOXICITY TEST, FRESHWATER DAPHNIDS
GUIDELINE OPPTS 850.1010

1. **CHEMICAL:** ECONEA Technical **PC Code No.:** 119093

2. **TEST MATERIAL:** CL 322,250 **Purity:** 92.6%
Lot or Batch No.: AC12395-43

3. **CITATION**

Authors:	Mark A. Cafarella
Title:	CL 322,250-Acute Toxicity to Water Fleas, (*Daphnia Magna*) Under Flow-Through Conditions
Study Completion Date:	June 28, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6151 Sponsor Protocol/Project No. AGR 925
MRID No.:	465960-08

4. **REVIEWED BY:**

Signature: **Date:** 3/30/06
David Bays, Microbiologist, RASSB, AD

5. **APPROVED BY:**

Signature: **Date:** 3/30/06
Norm Cook, Branch Chief, RASSB, AD

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Daphnia magna*
Age of Test Organism:	<24 hours
Definitive Test Duration:	48 hours (May 17-19, 2005)
Study Method:	Flow-through
Type of Concentrations:	Both (results based on mean-measured concentrations)

7. CONCLUSIONS

Results Synopsis:

48-Hour Values
EC_{50} = 0.51 mg a.i/L
95% confidence intervals = 0.42 to 0.61 mg a.i./L

NOEC= 0.25 mg a.i./L

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Minor guideline deviations that should not affect the results of the study

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1010:

- Size of the test organisms is not provided in the Study Report.
- Fortified laboratory well water was used in the study for the dilution water. The guidelines recommend surface or ground water, reconstituted water, deionized water, or dechlorinated tap water.
- The exact transition period was not reported.
- The coverage for the test containers was not provided in the Study Report.
- The guidelines recommend that the concentrations in replicates vary no more than ± 20%. The concentrations in the study were not measured in the replicates, but only in one sample for each treatment level and the control.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ *Daphnia magna* ▪ *D. pulex*	▪ *Daphnia magna* (p. 9)
Life Stage ▪ 1st instar (24 h)	▪ Yes, <24 hours. (p. 9)
All organisms from same source?	▪ Yes, Springborn Smithers culture facility. (p. 9)
Organisms approximately same size and age?	▪ Organisms were <24 hours. Size of the test organisms is not provided in the study report. (p. 12)
Signs of disease or injury?	▪ No signs of disease or injury. (p. 12)
Cultures ▪ Do not contain ephippia	▪ No ephippia was produced. (p. 12)
Acclimation Period ▪ Minimum 48-hrs	▪ Yes, 48 hours. (p. 12)
Feeding ▪ No feeding during study.	▪ Daphnids were not fed during the exposure. (p. 13)
Pretest Mortality ▪ No more than 20% mortality 48 hours prior to testing.	▪ No mortality was observed during the 48 hours prior to test initiation. (p. 12)

B. Test System

Guideline Criteria	Reported Information
Source of dilution water ▪ Surface or ground water, reconstituted water, deionized water, or dechlorinated tap water.	▪ Fortified laboratory well water. (p. 13)
Does water support test animals without observable signs of stress?	▪ Yes. (p. 13-14, 25)
Photoperiod ▪ 16-hr light and 8-hr dark with 15- to 30-minute transition period.	▪ 16-hr light and 8-hr dark and sudden transitions from light to dark and vice versa were avoided. (p. 13-14)
Test Chambers ▪ Material: Glass or stainless steel. ▪ Size: 250 ml. ▪ Loosely covered.	▪ Glass battery jars. (p. 15) ▪ 1600 mL. (p. 15) ▪ Coverage information not provided in report.
Water Temperature ▪ 20 ± 2°C	▪ 20 ± 2°C (p. 14, 18)

Dissolved Oxygen ▪ Between 60 and 105% saturation ▪ Do not aerate tests.	▪ 8.6 to 9.0 mg/L. Greater than 60% saturation. (p. 23) ▪ Aeration was not discussed in the report.
Total Hardness ▪ 180 mg/L as $CaCO_3$ (maximum).	▪ Ranged from 170 to 180 mg/L as $CaCO_3$ (p. 13)
Flow Rate (Flow-through Test) ▪ At least 5X volume of test chamber. ▪ No more than 10% variation between test chambers.	▪ Provided approximately six solution volume replacements per day. (p. 15) ▪ Flow-splitting accuracy was within 10% of the targeted delivery. (p. 15)
Solvents ▪ Not to exceed 100 mg/L.	▪ Use of solvents was not reported.

C. **Test Design**

Guideline Criteria	Reported Information
Range-Finding Test ▪ Widely-spaced concentrations (e.g., 1, 10, 100 mg/L). ▪ Minimum 5 daphnids per concentration.	▪ Concentrations used in study were based on the results of a chronic flow-through exposure of daphnids to CL322, 250 conducted at Springborn Smithers (Study No. 13751.6152). (p. 14) ▪ The protocol found in the report follows the guideline (p. 31)
Concentrations of Definitive Test ▪ Control & 5 or more treatment levels ▪ A geometric series with 1.5 to 2.0 progression. ▪ 2 or more replicates per dose. ▪ Static test: measured at beginning and end (minimum). ▪ Static renewal test: measured at beginning and end of each renewal period. ▪ Flow-through test: measured in each chamber at beginning of test and at 48 hours, and whenever malfunction detected. ▪ Concentrations in replicates vary no more than ± 20%.	▪ Yes (control, 0.31, 0.63, 1.3, 2.5, and 5.0 mg a.i./L). (p. 14) ▪ Yes. (p. 14) ▪ 2 replicates for each treatment level and the control. (p. 14) ▪ One samples from each treatment level, the control, and three quality control samples measured at 0 and 48 hours. (p. 16-17, 24) ▪ Concentrations were not measured in the replicates.
Number of Test Organisms ▪ Minimum 20/concentration, may be equally divided among containers ▪ Loading not to exceed 40 daphnids per liter of test solution in static system ▪ Loading in flow-through system dependent on flow rate.	▪ Yes. (10 daphnids per vessel and two replicates per treatment level). (p. 16) ▪ Daphnids were added no more than two at a time. Flow provided a 90% test solution replacement rate of approximately 9 hours. (p. 15)
Test organisms randomly or impartially assigned to test vessels?	▪ Yes. (p. 16)

Duration of Test ▪ 48 hours ▪ Each test chamber checked for immobilized daphnids at 24 and 48 hours.	▪ 48 hours. (p. 16) ▪ Yes. (p. 16)
Water Parameter Measurements ▪ Temp, DO and pH: measured at beginning and end of test in each chamber.	▪ Yes. Measured at 0, 24, and 48 hours in each chamber. (p. 16, 23)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	▪ Yes. (p. 3, 4)
Control Mortality ▪ Not more than 10%.	▪ No immobilization or adverse effects were observed in the control groups. (p. 19, 25)
Percent Recovery of Chemical	▪ Percent of nominal ranged from 79 to 110%. Percent recovery based on quality control samples ranged from 95.1 to 101%. (p. 19, 24)
Raw data included?	▪ Yes. (p. 23-25,)

Dose Response

Mortality:

Concentration (ppm)		Number of Organisms	Cumulative Number Dead	
Nominal (mg a.i./L)	Mean Measured (mg a.i./L)		Hour of Study	
			24	48
Control	Control	20	0	0
0.31	0.25	20	0	0
0.63	0.53	20	0	11
1.3	1.4	20	20	20
2.5	2.7	20	20	20
5.0	5.0	20	20	20

Statistical Results

Statistical Method:

The study reported that the mean measured concentrations tested and the corresponding immobilization data were used to estimate the 24- and 48-hour EC_{50} values and 95% confidence intervals. A computer program using binomial probability calculated the EC_{50} values and 95% confidence intervals.

It appears that the NOEC was determined by empirical analysis of the mortality data.

Results Synopsis:

24-Hour Values
EC_{50} = 0.86 mg a.i/L
95% confidence intervals = 0.53 to 1.4 mg a.i./L

48-Hour Values
EC_{50} = 0.50 mg a.i/L
95% confidence intervals = 0.25 to 0.53 mg a.i./L

NOEC= 0.25 mg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Versar calculated the 24- and 48-hour EC_{50} values for the mortality data using linear interpolation and the mean-measured concentrations.

The statistical computer program that determines the NOEC could not be performed because there were only two replicates in the study. The mortality data was empirically analyzed to determine the NOEC.

Results Verification Synopsis:

24-Hour Values
EC_{50} = 0.97 mg a.i/L
95% confidence intervals = 0.97 to 0.97 mg a.i./L

48-Hour Values
EC_{50} = 0.51 mg a.i/L
95% confidence intervals = 0.42 to 0.61 mg a.i./L

NOEC= 0.25 mg a.i./L

14. REVIEWER'S COMMENTS:

- Guideline deviations are shown in Section 9.
- The 24- and 48-hour EC_{50} values and 95% confidence intervals calculated by Versar were different than those reported by the study author. The differences may be due to the use of different statistical tests.

24-Hour EC_{25} Determination:

48-Hour EC_{25} Determination:

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)- (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** CL322,250 **Purity:** 92.6%

3. **CITATION:**

Author:	Arthur E. Putt
Title:	CL322,250 – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus)* Under Flow-though Conditions
Study Completion Date:	May 9, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1075
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6149 Janessen Study No. AGR 923
MRID No.:	MRID 465960-09

4. **REVIEWED BY:**

   Signature: **Date:** 3/30/06

   David Bays, Microbiologist, RASSB, AD

5. **APPROVED BY:**

   Signature: **Date:** 3/30/06

   Norm Cook, Branch Chief, RASSB, AD

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Lepomis macrochirus*
Age of Test Organism:	Not provided
Definitive Test Duration:	4 days, March 17-21, 2005
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean measured

7. **CONCLUSIONS**

   Results Synopsis:

96-hour LC_{50}: 1.2 mg a.i./L Confidence (95%) interval: 1.1-1.4 mg a.i./L
96-hour NOEC: 0.55 mg a.i./L

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Minor guideline deviations that should not affect the results of the study.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm with a fill volume of 6.8 L. Guidelines state that the aquaria should be 30 x 60 x 20 cm and have a fill volume of 15 to 30 L of solution.
- The biomass loading was 0.35 g/L/day instead of the guideline stipulation of 1 g/L/day.
- The dissolved oxygen level dropped below the 75% guideline stipulation in two replicate chambers of the treatments.
- No statement was made as to the signs of disease 48-hours prior to testing.
- Fish were not noted as either being or not being from the same class year.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • Preferred species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*)	• Bluegill sunfish (*Lepomis macrochirus*)
Mean Weight • 0.5-5 g	• Mean: 1.8 g (p. 9) • Range: 0.90-3.1 g (p. 9)
Mean Standard Length • Longest not > 2x shortest	• Yes, Mean= 49 mm and ranged form 42-60 mm (p. 9)

Supplier	Osage Catfisheries Osage Beach, Missouri (p. 12)
All fish from same source?	Yes (p. 12)
All fish from the same year class?	Not provided

B. Source/Acclimatiou

Guideline Criteria	Reported Information
Acclimation Period • Minimum 14 days	• Yes (p.13)
Wild caught organisms were quarantined for 7 days?	• Not applicable
Were there signs of disease or injury?	• Information not provided (p.13)
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	• No sign of mortality 48-hours prior to testing, no other observations provided. (p. 13)
Feeding • No feeding during the study	• Feeding was not conducted 48 hours prior to testing or during testing. (p. 13)
Pretest Mortality • No more than 3% mortality 48 hours prior to testing	• The mortality rate was 0% 48 hours prior to test initiation. (p.13)

C. Test System

Guideline Criteria	Reported Information
Source of dilution water • Soft reconstituted water or water from a natural source, not dechlorinated tap water	• Yes, well water was utilized. (p. 13)
Does water support test animals without observable signs of stress?	• Yes, freshwater organisms have survived and reproduced for generations in the well water. (p. 13)
Water Temperature • 12°C for cold water species • 17°C or 22°C for warm water species	• Test temperatures were from 22 to 23°C (p. 19)
pH • Prefer 7.2 to 7.6	• pH ranged from 7.3 to 7.8 (p. 23)
Dissolved Oxygen • Flow-through: >75%	• In replicate A of the 0.58 mg/L treatment, the dissolved oxygen concentration dropped to 72% but raised to 77% by scraping microbial growth from aquarium. In replicate B of the 0.97 mg/L treatment, the dissolved oxygen concentration was found to be 73% at test termination. All other replicates were above 75% saturation. (p.19)

Guideline Criteria	Reported Information
Total Hardness • Prefer 40 to 180 mg/L as $CaCO_3$	• Total hardness as calcium carbonate: 52 mg/L. (p.13)
Test Aquaria • Material: Glass or stainless steel • Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm • Fill volume: 15-30 L of solution	• Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm (p. 14 and 15) • Fill Volume: 6.8 L (p.15)
Type of Dilution System • Must provide reproducible supply of toxicant	• Yes, the dilution system was in operation for seven days prior to testing to ensure constant test substance placement. (p.15)
Flow Rate • Consistent flow rate of 5-10 vol/24 hours • Meter systems calibrated before study and checked twice daily during test period	• Constant flow rate at 7.7 volume replacements/ day. • The system was calibrated seven days prior to test initiation and visually inspected twice a day. (p.15)
Biomass Loading Rate • Static: 0.8 g/L at 17°C, 0.5 g/L at > 17°C • Flow-through: 1 g/L/day	• Biomass loading 0.35 g/L/day (p.16)
Photoperiod • 16 hours light, 8 hours dark	• 16 hours light, 8 hours dark (p. 14 and 12)
Solvents • Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	• Acetone: 0.10 mL/L (p. 14 and 15)

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test • If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	• Preliminary test conducted • The nominal concentrations were 0.58, 0.97, 1.6, 2.7, and 4.5 mg a.i./L. • Five test organisms per treatment level. • After 96 hours, 100% mortality in 1.6, 2.7, and 4.5 mg/L treatment levels. No mortality in the 0.58 and 0.97 mg/L treatments. (p. 18 and 19)
Nominal Concentrations of Definitive Test • Control & 5 treatment levels • Dosage should be 60% of the next highest concentration • Concentrations should be in a geometric series	• Control, solvent control, and at 0.35, 0.58, 0.97, 1.6, and 2.7 mg a.i./L. • Nominal concentrations were approximately 60% of the next highest. (p. 15) • Concentrations were in a geometric series.
Number of Test Organisms • Minimum 10/level • May be divided among containers	• 20/level, two test aquaria per treatment level. (p. 16)

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	• Selected impartially (p. 16)
Biological observations made every 24 hours?	Yes, at initiation, 24, 48, 72, and 96 hours. (p. 16)
Water Parameter Measurements • Temperature: Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1 C • DO and pH: Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	• Temperature, DO, and pH measurements were conducted for all treatment levels and aquaria daily. • Test solution temperature continuously measured during test in replicate A of control (p. 16) • Temperatures did not vary more than a degree. (p. 19)
Chemical Analysis • Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	• Prior to initiation, samples taken from replicates of high, medium low and control treatment levels and analyzed (p.17) • Sample of stock solution analyzed during pre-test period (p. 17) • During study, one water sample from 1 replicate of each treatment level and controls collected and analyzed at 0-hr and 96-hr (p. 17) • Samples removed from alternate replicates and initiation and termination (p. 17)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Percent Recovery of Chemical from Chemical Analysis	Yes, the recovery was between 92-97% (p. 24)
Control Mortality • Not more than 10% control organisms may die or show abnormal behavior.	• No mortality was seen in control or solvent control. (p. 25)
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes, organisms were noted to be dead, lethargic, or dark in coloration. (p. 25)

Dose Response

Mortality

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Number of Fish at Test Initiation (Rep A / Rep B)	Number of Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Control	Control	10/10	0/0	0/0	0/0	0/0
Solvent Control	Solvent Control	10/10	0/0	0/0	0/0	0/0
0.35	0.32	10/10	0/0	0/0	0/0	0/0
0.58	0.55	10/10	0/0	0/0	0/0	0/0
0.97	0.92	10/10	0/0	0/0	1/0	1/0
1.6	1.6	10/10	0/0	6/7	7/7	9/10
2.7	2.5	10/10	10/10	10/10	10/10	10/10

Symptoms

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Symptoms			
		24 hour	48 hour	72 hour	96 hour
Control	Control	0	0	0	0
Solvent Control	Solvent Control	0	0	0	0
0.35	0.32	0	0	0	0
0.58	0.55	0	0	0	0
0.97	0.92	0	0	0	0
1.6	1.6	0	2[a]	2[a,b]	1[a]
2.7	2.5	0	0	0	0

a Observed to be lethargic
b Observed to be dark in coloration

Statistical Results

Statistical Method: The 24- and 48-hour LC_{50}'s were estimated using binomial probability. The 72- and 96-hour LC_{50}'s were estimated using probit analysis. The NOEC was estimated by visual inspection. (p. 20)

Results Synopsis:

24-hour LC_{50}: 2.0 mg a.i./L Confidence (95%) interval: 1.6-2.5 mg a.i./L
48-hour LC_{50}: 1.5 mg a.i./L Confidence (95%) interval: 0.92-2.5 mg a.i./L
72-hour LC_{50}: 1.4 mg a.i./L Confidence (95%) interval: 1.2-1.6 mg a.i./L
96-hour LC_{50}: 1.2 mg a.i./L Confidence (95%) interval: 1.1-1.4 mg a.i./L
96-hour NOEC: 0.55 mg a.i./L

13. **VERIFICATION OF STATISTICAL RESULTS**
Versar did not verify results.

14. **REVIEWER'S COMMENTS:**
No additional comments.

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)- (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** CL322,250 **Purity:** 92.6%

3. **CITATION:**

Author:	Arthur E. Putt
Title:	CL322,250 – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-though Conditions
Study Completion Date:	April 26, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1075
Sponsor:	Janssen Pharmaceutical N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6150 Janessen Study No. AGR 924
MRID No.:	MRID 465960-10

4. **REVIEWED BY:**

   Signatnre: **Date:** 3/30/06

   David Bays, Microbiologist, RASSB, AD

5. **APPROVED BY:**

   Signature: **Date:** 3/30/06

   Norm Cook, Branch Chief, RASSB, AD

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Oncorhynchus mykiss*
Age of Test Organism:	Not provided; used juveniles
Definitive Test Duration:	4 days, March 4-8, 2005
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean measured

7. **CONCLUSIONS**

Results Synopsis:

96-hour LC_{50}: 520 µg a.i./L (Confidence (95%) interval: 320-870 µg a.i./L)
96-hour NOEC: 320 µg a.i./L

8. **ADEQUACY OF THE STUDY**

A. Classification: Core

B. Rationale: Minor guideline deviations that should not affect the results of the study

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS:**

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm with a fill volume of 6.8 L were used. Guidelines state that the aquaria should be 30 x 60 x 20 cm and have a fill volume of 15 to 30 L of solution.
- The biomass loading was 0.15 g/L/day, instead of the guideline stipulation of 1 g/L/day.
- No statement was made as to the signs of disease 48-hours prior to testing.
- Fish were not noted as either being or not being from the same class year.

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species • Preferred species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*)	• Rainbow trout (*Oncorhynchus mykiss*)
Mean Weight • 0.5-5 g	• Mean: 0.79 g (p. 9) • Range: 0.46-1.17 g (p. 9)

Mean Standard Length • Longest not > 2x shortest	• Yes, Mean= 44 mm and ranged form 36-49 mm (p. 9)
Supplier	Troutlodge, Inc. Sumner, Washington (p. 12)
All fish from same source?	Yes (p. 12)
All fish from the same year class?	Not provided

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period • Minimum 14 days	• Yes (p.13)
Wild caught organisms were quarantined for 7 days?	• Not applicable
Were there signs of disease or injury?	• Information not provided (p.13)
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	• No sign of mortality 48-hours prior to testing, no other observations provided. (p. 13)
Feeding • No feeding during the study	• Feeding was not conducted 48 hours prior to testing or during testing. (p. 13)
Pretest Mortality • No more than 3% mortality 48 hours prior to testing	• The mortality rate was 0% 48 hours prior to test initiation. (p.13)

C. Test System

Guideline Criteria	Reported Information
Source of dilution water • Soft reconstituted water or water from a natural source, not dechlorinated tap water	• Yes, well water was utilized. (p. 13)
Does water support test animals without observable signs of stress?	• Yes, freshwater organisms have survived and reproduced for generations in the well water. (p. 13)
Water Temperature • 12°C for cold water species • 17°C or 22°C for warm water species	• Test temperatures were from 12 to 13°C (p. 24)
pH • Prefer 7.2 to 7.6	• pH ranged from 7.5 to 7.7 (p. 24)
Dissolved Oxygen • Flow-through: >75%	• DO concentrations were above 75% throughout the test. (p.24)

Guideline Criteria	Reported Information
Total Hardness • Prefer 40 to 180 mg/L as $CaCO_3$	• Total hardness as calcium carbonate: 56 mg/L. (p.13)
Test Aquaria • Material: Glass or stainless steel • Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm • Fill volume: 15-30 L of solution	• Glass aquaria with silicone sealant measuring 30 x 15 x 20 cm (p. 14 and 15) • Fill Volume: 6.8 L (p.15)
Type of Dilution System • Must provide reproducible supply of toxicant	• Yes (p.26)
Flow Rate • Consistent flow rate of 5-10 vol/24 hours • Meter systems calibrated before study and checked twice daily during test period	• Constant flow rate at 7.9 vol. replacements/day (p. 15). • The dilution system was calibrated prior to initiation and visually inspected twice a day. (p.15)
Biomass Loading Rate • Static: 0.8 g/L at 17°C, 0.5 g/L at > 17°C • Flow-through: 1 g/L/day	• Biomass loading 0.15 g/L/day (p.16)
Photoperiod • 16 hours light, 8 hours dark	• 16 hours light, 8 hours dark (p. 14 and 12)
Solvents • Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	• Acetone: 0.10 mL/L (p. 14 and 15)

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test • If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	• Preliminary test conducted • The nominal concentrations were 0.52, 0.86, 1.4, 2.4, and 4.0 mg a.i./L. • Five test organisms per treatment level. • After 96 hours, 100% mortality in 0.86, 1.4, 2.4, and 4.0 lm/L treatment levels. Mortality rate of 80% was noted at the lowest treatment level, 0.52 mg/L. (p. 18 and 19) • 2nd test conducted with nominal concentrations of 110, 310, and 810 ug/L and 5 test organisms per treatment level • after 2 hours of exposure, 100% mortality in 860 ug/L treatment level; no mortality in other treatment levels.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test • Control & 5 treatment levels • Dosage should be 60% of the next highest concentration • Concentrations should be in a geometric series	• Control, solvent control, and at 860, 520, 310, 190, and 110 µg a.i./L • Nominal concentrations were approximately 60% of the next highest. (p. 15) • Concentrations were in a geometric series.
Number of Test Organisms • Minimum 10/level • May be divided among containers	• 20/level, two test aquaria per treatment level. (p. 16)
Test organisms randomly or impartially assigned to test vessels?	• Selected impartially (p. 16)
Biological observations made every 24 hours?	Yes, at initiation, 24, 48, 72, and 96 hours. Observed for signs of mortality, with dead fish being removed, and adverse effects. (p. 16)
Water Parameter Measurements • Temperature: Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1 C • DO and pH: Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	• Temperature, DO, and pH measurements were conducted for all treatment levels and aquaria daily. • Test solution temperature continuously measured during test in replicate A of control (p. 16) • Temperatures did not vary more than a degree. (p. 19)
Chemical Analysis • Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	• Prior to initiation, samples taken from replicates of high, medium low and control treatment levels and analyzed (p.17) • Sample of stock solution analyzed during pre-test period (p. 17) • During study, one water sample from 1 replicate of each treatment level and controls collected and analyzed at 0-hr and 96-hr (p. 17) • Samples removed from alternate replicates and initiation and termination (p. 17)

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Percent Recovery of Chemical from Chemical Analysis	Yes, 100% of the chemical at all treatment levels was recovered at test termination.

Guideline Criteria	Reported Information
Control Mortality • Not more than 10% control organisms may die or show abnormal behavior.	• No mortality was seen in control or solvent control. (p. 25)
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes, organisms were noted to be dead, lethargic, or dark in coloration. (p. 26)

Dose Response

Mortality

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Number of Fish at Test Initiation (Rep A/ Rep B)	Number of Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Control	Control	10/10	0/0	0/0	0/0	0/0
Solvent Control	Solvent Control	10/10	0/0	0/0	0/0	0/0
110	110	10/10	0/0	0/0	0/0	0/0
190	190	10/10	0/0	0/0	0/0	0/0
310	320	10/10	0/0	0/0	0/0	0/0
520	540	10/10	0/3	2/4	5/6	5/6
860	870	10/10	10/10	10/10	10/10	10/10

Symptoms

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Symptoms			
		24 hour	48 hour	72 hour	96 hour
Control	Control	0	0	0	0
Solvent Control	Solvent Control	0	0	0	0
110	110	0	0	0	0
190	190	0	0	0	0
310	320	0	0	0	0
520	540	1[c] & 1[b]	1[c]	1[c]	1[c] & 2[a]
860	870	0	0	0	O

a Observed to be lethargic
b Observed to be dark in coloration
c Observed to lethargic and dark in coloration

Statistical Results

Statistical Method: All LC_{50} values (24, 48, 72, and 96-hr) and confidence intervals were measured by binominal probability. (p. 27)

Results Synopsis:

24-hour LC_{50}:	640 µg a.i./L	Confidence (95%) interval:	540-870 µg a.i./L
48-hour LC_{50}:	600 µg a.i./L	Confidence (95%) interval:	320-870 µg a.i./L
72-hour LC_{50}:	520 µg a.i./L	Confidence (95%) interval:	320-870 µg a.i./L
96-hour LC_{50}:	520 µg a.i./L	Confidence (95%) interval:	320-870 µg a.i./L
96-hour NOEC:	320 µg a.i./L		

13. **VERIFICATION OF STATISTICAL RESULTS**
Versar did not verify results.

14. **REVIEWER'S COMMENTS:**
No additional comments.

DATA EVALUATION RECORD
DAPHNID CHRONIC TOXICITY TEST
GUIDELINE OPPTS 850.1300

1. **CHEMICAL:** ECONEA Technical **PC Code No.:** 119093

2. **TEST MATERIAL:** CL322, 250 **Purity:** 92.6%
Lot or Batch No.: AC12395-43

3. **CITATION**

Authors:	Mark A. Cafarella
Title:	CL322,250 - Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Flow-Through Conditions
Study Completion Date:	June 27, 2005
Report Date:	June 27, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751-6152 Sponsor Protocol/Project No. AGR 926
MRID No.:	465960-11

4. **REVIEWED BY:**

Signature: **Date: 10/12/06**
David Bays, RSSAB, AD (7510C)

5. **APPROVED BY:**

Signature: **Date: 10/12/06**
Norm Cook, Branch Chief, RSSAB

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Daphnia magna*
Age of Test Organism:	≤ 24-hours old
Definitive Test Duration:	21 days
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean-measured

7. CONCLUSIONS

Results Synopsis:

Reproduction and Growth
NOEC = 0.30 mg a.i./L
LOEC = 0.54 mg a.i./L
MATC = 0.40 mg a.i./L

Survival
EC_{50} value = 1.2 mg a.i./L
95% confidence interval = 0.86 to 2.0 mg a.i./L

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Scientifically Acceptable Study.

C. Repairability: Upgraded to Core upon resolution of points 1,3, and 5 below and submission of missing raw data on reproduction. (MRIDs 46917901, and supplemental report MRID 46917902)

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.1300:

1.) The guidelines state to maintain cultures in 100% dilution water at test conditions (temperature, diet, background colors, and light intensity). However, the study reports that the culture solution temperature was maintained at 20 ± 2 °C, but test solution temperature ranged from 20 to 21 °C. In addition, there was a culture light intensity of 1200 to 1500 lux, but a test light intensity of 440 to 910 lux. **(similar and as evidenced by the excellent The environmental conditions maintained in culture and test are performance of control daphnids in the study ~~that~~ any differences are considered inconsequential)**

2.) The study reports that during culture daphnids were fed 2.0 mL of green algae and 0.5 mL of YCT suspension per test vessel once daily. During the definitive test, feeding consisted of 3.0 mL of algal suspension and 1.0 mL of YCT suspension per test vessel three times daily. This does not follow the guideline recommendation that during the test daphnids should be fed the same diet and at the same frequency as cultures. **(Not addressed by the registrant)**

3.) The study did not report water quality parameters for particulates, un-ionizable ammonia, residual chlorine, total organophosphorus pesticides 50 ng/L, total organochlorine pesticides plus PCBs or organic chlorine. **(The dilution water was analyzed periodically for the presence of pesticides, PCB and toxic metals. The total suspended solids concentration for the dilution water was 0.30 mg/L)**

4.) The study did not report if test equipment and test chambers were cleaned before each use using good laboratory practices. **(Not addressed by the registrant)**

5.) The study did not report any information about aeration or if it was done before the addition of the test substance. **(No aeration was employed during the study, but dilution water was aerated prior to delivery to the exposure system. This, combined with the presence of photosynthesizing algae (as a food source) in the test vessels, provided acceptable dissolved oxygen levels throughout the duration of the study)**

6.) The study reported that a range-finding test was conducted with exposure to nominal CL322,250 treatment levels of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.i./L and a control under flow-through conditions. The guidelines recommend exposure to a series of widely spaced concentrations of the test chemical (e.g., 1, 10, 100 mg/L), usually under static conditions.

(Raw data on reproduction was provided by the testing lab in the form of a supplemental report and was found to be acceptable. MRID# - 469179-02)

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species •*Daphnia magna* •*D. pulex*	• *Daphnia magna*
Life Stage • First instar, #24 hours old.	• ≤ 24-hours old (p. 9)
Source • Daphnids should be cultured at the test facility and originate from same culture population.	• Springborn Smithers culture (p. 9, 13)

Culturing: X Source of initial stock and culturing techniques described. X Do not use daphnids if: X - Cultures contain ephippia. X - Adults in cultures do not produce young before day 12. X - More than 20% of the culture stock die during the 2 days preceding the test. X - Adults in the culture do not produce an average of at least three young per day over the 7-day period prior to test. X - Daphnids have been used in any portion of a previous test, either in a treatment or in a control.	• Yes. (p. 13) Adult daphnids use to produce offspring: • Did not contain ephippia (p. 13) • Produced offspring prior to being 12 days old (p. 13) • Survival 48 hours prior to test initiation was 100% (p. 13) • Produced an average of 12.5 offspring per female per day seven days prior to test initiation (p. 13) • Were not used in any portion of a previous test (p. 13)
Acclimation X Acclimate at least 48 hours prior to start of test. X Maintain in 100% dilution water at test conditions (temperature, diet, background colors, and light intensity). X Should be fed same food as used during definitive test	• Yes. (p. 13) • Culture solution temperature at 20 ± 2 °C, but test solution temperature ranged from 20 to 21 °C. Culture light intensity of 1200 to 1500 lux, but test light intensity of 440 to 910 lux. Test and culture photoperiod of 16 hours light and 8 hours darkness. (p. 13-15) • See below for details on feeding.
Feeding • During test, daphnids should be fed same diet and at same frequency as cultures. • Suggested rates: 5 to 7 mg/L of dilution water or test solution (automatic); 15 mg (dry weight)/L (manual).	• Culture feeding: 2.0 mL of green algae and 0.5 mL of YCT suspension per test vessel once daily. (p. 14) • Test feeding: 3.0 mL of algal suspension and 1.0 mL of YCT suspension per test vessel three times daily. (p.14)

B. Test System

Guideline Criteria	Reported Information
System • Static-renewal: dilution water completely replaced at least once every 3 days. • Flow-through: • - Calibrate system before each test. • - Check general operation at least twice during test. • - 24-hour flow through a test chamber should equal at least 5x volume of chamber. • - Flow rate should not vary by more than 10% from one chamber to another.	• Diluter system calibrated prior to test initiation and confirmed at test termination. (p. 16) • Function of the diluter system was monitored daily and a visual check of the operation was performed twice each day. (p. 16) • Test solutions were delivered to the exposure vessels at an approximate rate of 6 test vessel volumes per 24-hour period. (p. 17) • Flow-splitting accuracy was within 10% of the targeted delivery. (p. 16)

Dilution Water X Surface or ground water, reconstituted water, (deionized) water, or dechlorinated tap water acceptable. X Water quality parameters (maximum): X Particulates 20 mg/L X TOC 2 mg/L or COD 5 mg/L X Un-ionizable ammonia 20Φg/L X Residual chlorine <3 µg/L X Total organophosphorus pesticides 50 ng/L X Total organochlorine pesticides plus PCBs (50 ng/L) or organic chlorine 25 ng/L X Water quality should be tested at least twice per year. X If diluent is groundwater or surface water, conductivity and TOC or COD should be measured.	• Fortified and filtered well water. (p. 14) • Water quality parameters were measured on each batch of fortified water prior to use. Fortified water was discarded if not used within 14 days of preparation. (p. 14) • TOC = 0.29 mg/L. Specific conductivity of 500 µmhos/cm. (p. 14)
Photoperiod • 16-hr light/8-hr dark	• Yes. (p. 15)
Test Chambers • 250-mL beakers or other suitable containers. • Loosely covered to reduce loss of test solution or dilution water due to evaporation and to minimize entry of dust or other particulates. • Test equipment and test chambers should be cleaned before each use using good laboratory practices. • For flow-through tests: daphnids can be in glass or stainless steel containers with stainless steel or nylon bottoms suspended in test chamber to ensure test solution flows regularly into and out of containers and daphnids are always submerged in at least 5 cm of test solution.	• 1.6-L glass battery jars. (p. 16) • Loosely covered with plastic. (p. 16) • Cleaning of test equipment and test chambers not reported. • Exposure solutions drained from each vessel through two 2-cm holes approximately 15 cm from the bottom of the glass jars which maintained the test solution volume of 1.4 L. Drain holes were covered with a Nitex 40-mesh screen. (p. 16)
Temperature • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations. • 20 ± 1EC	• Measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). (p. 18) • 20-21EC. (p. 23, 30)
Dissolved Oxygen • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations. • Between 60 and 105 percent saturation. • Aeration should be done before addition of test substance.	• Measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). (p. 18) • Dissolved oxygen = 8.1 to 9.9 mg/L (91% to 109%) (p. 23, 30) • No aeration was employed.

pH • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations.	• Measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). (p. 18) • pH = 8.1 to 8.3. (p. 23, 30)
Solvents and Carriers • Concentration of carrier #0.1 mL/L. • Triethylene glycol and dimethyl formamide preferred solvents, but acetone or ethanol can be used if necessary.	• No use of solvent or carrier reported.

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Should be conducted to establish test solution concentrations in definitive test. • Exposure to a series of widely spaced concentrations of the test chemical (e.g., 1, 10, 100 mg/L), usually under static conditions. • Minimum of five daphnids should be exposed to each concentration of test substance. • Exposure period may be shortened if suitable data can be obtained in less time. • No replicates required and nominal concentrations of chemical acceptable.	• Range-finding test conducted. (p. 22) • Exposure to nominal CL322,250 treatment levels of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.i./L and a control under flow-through conditions. (p. 22) • Two replicate vessels (10 daphnids per vessel) were established for each concentration and four replicate vessels (10 daphnids per vessel) were established for the control. (p. 22)
Doses • Five or more concentration in a geometric series with a 1.5 to 2.0 progression (e.g., 2, 4, 8, 16, 32, and 64 mg/L).	• Nominal concentrations= control, 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.i./L (p. 23) • Mean measured concentrations = control, 0.11, 0.30, 0.54, 0.86, and 2.0 mg a.i./L. (p. 24)
Test Substance Concentration • At minimum, concentration of test chemical should be measured in each chamber before the test and on days 7, 14, and 21 of the test, and in at least one appropriate chamber whenever a malfunction is detected. • Concentrations of test substance in replicate test chambers should not vary more than ± 20%.	• Twice prior to initiation, samples were taken from both replicates of high, medium, low and control treatment levels and analyzed (p.19) • During study, water samples were removed from both replicates of each treatment level and the control and analyzed on test days 0, 7, 14, and 21(p. 19) • Concentrations did not vary more than ± 20%. (p. 31)

Controls X Controls should consist of same dilution water, conditions, and procedures, and daphnids. X Negative and/or solvent	• Yes. (p. 16, 17) • Negative control.
Replicates Per Dose • Equal number of daphnids in 2 or more replicates per dose (flow-through) • One daphnid each in 10 or more replicates per dose (static-renewal).	• 10 organisms per replicate vessel. (p. 17) • 2 replicates for each of 5 concentrations and the control. (p. 17)
Number of Organisms: • Minimum of 20 daphnids per concentration (flow-through). • Minimum of 10 daphnids per concentration (static-renewal). • Test organisms randomly or impartially placed in the test chambers. • Loading should not exceed 40 daphnids per liter of test solution in static-renewal system. • Loading in flow-through test varies depending on flow rate of test solution.	• Yes. (p. 17) • Daphnids were impartially added. (p. 17)
Duration of Test • 21 days	• Yes. (p. 9)
Observation of Daphnids • Daphnids in the test chambers observed on day 21 of the test. • Offspring should be counted and removed from the test chambers every 2 or 3 days. • Abnormal behavior or appearance reported.	• The number of immobilized offspring and adult daphnids and observations of abnormal behavior were recorded on days 0, 2, 4, 6, 7, 10, 13, 14, 17, 20 and 21. (p. 17) • Assessments of offspring released were determined beginning on day 8 and three times per week through day 21. (p. 17) • The total body length and dry weight of each surviving adult daphnid was measured on day 21. (p. 17-18)
Test Endpoints Measured • Number of daphnids immobilized (EC_{50} values and 95% C.I.) • Number of young per adult. • MATC determined for most sensitive endpoint.	• EC_{50} value = 1.2 mg a.i./L and 95% confidence interval = 0.86 to 2.0 mg a.i./L (p. 10) • Mean cumulative offspring per female. (p. 33) • MATC = 0.40 mg a.i./L (p. 10)
Growth • Determined by measuring total body length or dry weight (both preferred).	• The total body length and dry weight of each surviving adult daphnid was measured on day 21. (p. 18, 34)

Validity of Test Test is only valid if: X Less than 20% of the control should be immobilized, stressed, or diseased at the end of the study. X Each control daphnid should have produced at least 60 young after 21 days. X The controls should not produce any ephippia.	• Raw immobilization, stress, and disease data could not be found in the report. 95% survival of parental daphnids in control vessel after 21 days. (p. 32) • Mean cumulative number of offspring per control female was 154 after 21 days. (p. 24, 33) • Registrant has confirmed that if no ephippia are present, they are not mentioned in the study report.

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes. (p. 3, 4)
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	• Yes. (cover page, p. 9)
Growth of the daphnids determined by total body length or body weight?	• Yes, weight and length. (p. 18, 34)
Source of test material, lot number, composition, known chemical and physical properties, and any carriers or other additives used and their concentrations reported?	• Source, batch number, and purity reported. (p. 12, 13) • Chemical/physical properties not reported.
Source of the dilution water, its chemical characteristics (e.g. conductivity, hardness, pH), and a description of any pretreatment reported?	• Yes. (p. 14-15)
Detailed information about the daphnids provided?	• Yes. (p. 13-14)
Description of the test chambers provided?	• Yes. (p. 16-17)
Concentration of the test substance in the test chambers at the designated times provided?	• Yes, at days 0, 7, 14, and 21. (p. 31)
Number and percentage of organisms that showed any adverse effect reported?	• Provided in the study report. (See pages 24-31 of the supplemental report)
Cumulative adult and offspring immobilization values, progeny produced, the time to first brood, the number offspring per adult, and growth of surviving adults measured?	• Cumulative mean percent survival, mean cumulative number of offspring per female, and mean total body lengths and dry weights reported. (p. 32-34). • Time to first brood also reported. (p. 25)
All chemical analysis (of water quality) and test substance concentrations, including methods, method validation, and reagents reported?	• Yes. (Appendix 2)

Data records of the culture, acclimation, and test temperature provided?	• Data records of test temperature provided (p. 30) • Data records of the culture and acclimation period not provided.
Deviations from the test guideline provided and anything unusual about test (e.g., dilution failure, temperature fluctuations) reported?	• Protocol deviations provided. (p. 27)
MATC reported and statistical methods employed reported?	• Yes. (p. 10, 20-22)
Concentration-response curves utilizing average test substance concentration and adult immobilization data at 21 days provided?	• No
EC50 value based on adult immobilization calculated using the average measured concentration of the test substance?	• Yes. (p. 21)
Raw data included:	• Summary data only. (p. 30-34)
Statistical methods reported:	• Yes. (p. 20-21)

Dose Response

Test Group	Nominal Concentration (mg/L)	Mean Concentration (mg/L)	Mortality (%)	Mean No. Offspring Produced Over 21 Days	Mean Dry Weight at Day 21 (mg) (SD)	Mean Total Body Length at Day 21 (mm) (SD)
Control	0	0	5	154	1.01 (0.16)	4.65 (0.17)
1	0.13	0.11	0	148	0.91 (0.17)	4.59 (0.16)
2	0.25	0.30	5	160	0.97 (0.17)	4.67 (0.14)
3	0.50	0.34	0	135	0.84 (0.14)	4.45 (0.17)
4	1.0	0.86	5	104	0.70 (0.16)	4.21 (0.20)
5	2.0*	2.0*	100	0	NA	NA

*Treatment level excluded from statistical analysis due to statistically significant reduction in survival.

Statistical Results

Statistical Method: Survival, reproduction and growth data were analyzed to determine if there were any statistically significant treatment effects. Analyses were performed using the mean replicate organism response in each treatment group rather than individual response values. Survival, reproduction and grow data were checked for normality with the Shapiro-Wilks' Test and for homogeneity of variance using the Barlett's test. Survival and weight data were also analyzed for homogeneity of variance by Hartley's Test.

The NOECs and LOECs were calculated based on daphnid reproduction and growth. Statistical analysis for reproduction and total body length was performed using Williams' Test. Statistical analysis for dry weight was performed using Dunnett's Test. The MATC was calculated based on the geometric mean of the NOECs and LOECs. The 2.0 mg/L treatment level was excluded from statistical analysis of reproduction and growth data due to the statistically significant reduction in survival.

Based on daphnid survival, the 21-day EC_{50} value was calculated by binomial probability.

Results Synopsis:
Reproduction and Growth
NOEC = 0.30 mg a.i./L
LOEC = 0.54 mg a.i./L

MATC = 0.40 mg a.i./L

Survival
EC_{50} value = 1.2 mg a.i./L
95% confidence interval = 0.86 to 2.0 mg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:

NOEC/LOEC
Total body length and dry weight data were analyzed to determine if there were any statistically significant treatment effects. The data were first checked for normality using the Shapiro-Wilks test and for homogeneity of variances using Bartlett's test. The length data passed for both normality and homogeneity of variance. The dry weight data passed for normality but failed for homogeneity because of zero variance.

The NOECs and LOECs were then determined using Dunnett's test. For total body length, the test determined a significant difference in the 0.54 and 0.86 mg a.i./L treatment levels compared to the control. For dry weight, the test indicated a significant difference in mean dry weight in the 0.11, 0.54 and 0.86 mg a.i./L treatment levels compared to the control. The study reported the same findings, however, the observed reduction in dry weight at the 0.11 mg a.i./L treatment level was not considered to be biologically relevant due to the lack of a similar reduction at the next highest treatment level. Therefore, the study's results of a NOEC of 0.30 mg a.i./L and a LOEC of 0.54 mg a.i./L for growth were verified. The 2.0 mg/L treatment level was excluded from statistical analysis of reproduction and growth data due to the statistically significant reduction in survival.

MATC
The MATC was calculated by taking the geometric mean of the NOEC and LOEC. The MATC was determined to be 0.40 mg a.i./L and matched the result stated in the study report. (See MRID 46917902)

Results Verification Synopsis:
Growth
NOEC = 0.30 mg a.i./L

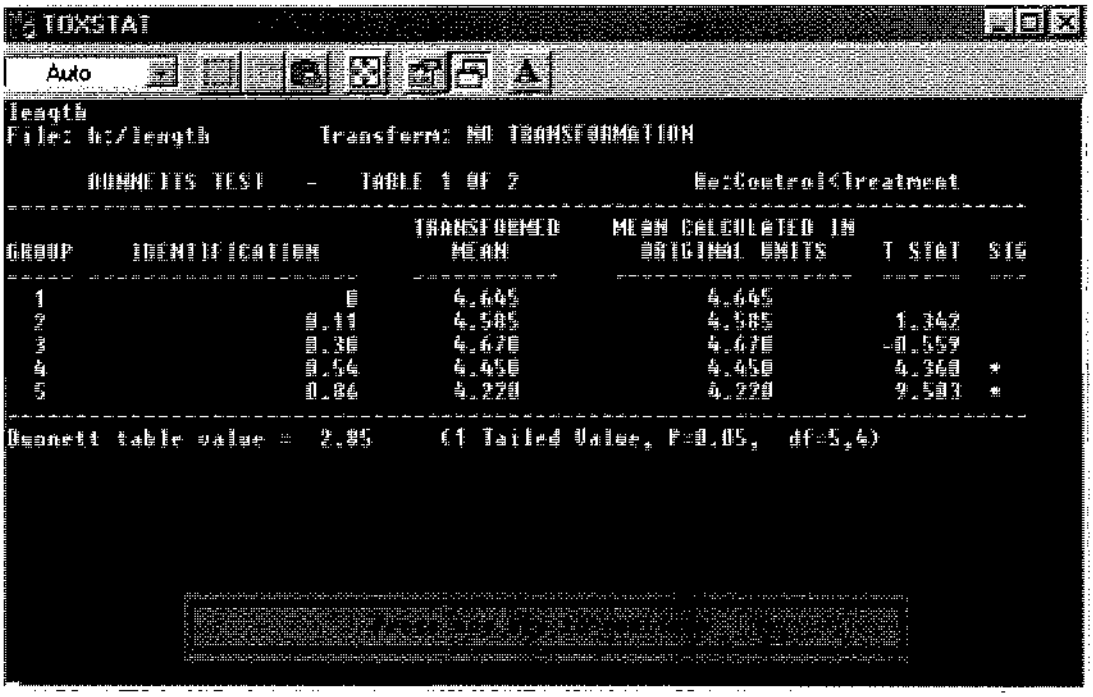
LOEC = 0.54 mg a.i./L

MATC = 0.40 mg a.i./L

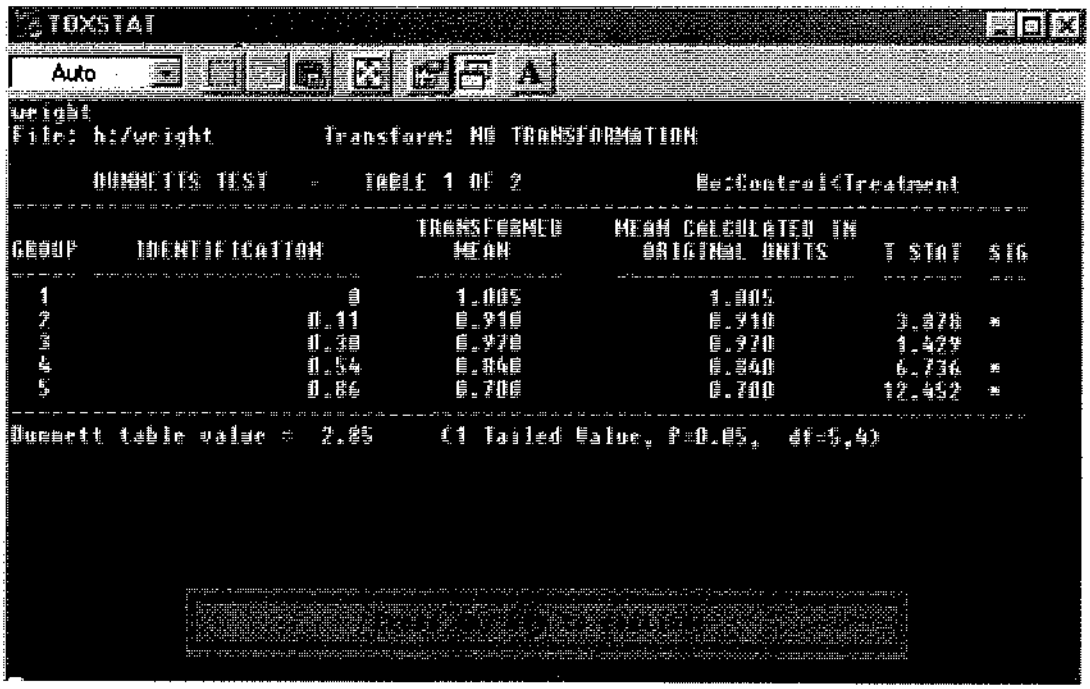
14. REVIEWER'S COMMENTS:

- Guideline deviations ~~are~~ were addressed by the registrant in MRIDS 46917901 and 46917902.

Daphnia Total Body Length-NOEC/LOEC



Daphnia Dry Weight-NOEC/LOEC



DP Barcode: 321452 MRID No: 465960-11

DATA EVALUATION RECORD
DAPHNID CHRONIC TOXICITY TEST
GUIDELINE OPPTS 850.1300

1. **CHEMICAL:** ECONEA Technical **PC Code No.:** 119093

2. **TEST MATERIAL:** CL322, 250 **Purity:** 92.6%
Lot or Batch No.: AC12395-43

3. **CITATION**

Authors:	Mark A. Cafarella
Title:	CL322,250 - Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Flow-Through Conditions
Study Completion Date:	June 27, 2005
Report Date:	June 27, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751-6152 Sponsor Protocol/Project No. AGR 926
MRID No.:	465960-11

4. **REVIEWED BY:**
Signature: [signature], Agronomist RASSB/AD **Date:** 4/11/06

5. **APPROVED BY:**
Signature: [signature] **Date:** 4/17/06

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Daphnia magna*
Age of Test Organism:	≤ 24-hours old
Definitive Test Duration:	21 days
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean-measured

7. CONCLUSIONS

Results Synopsis:

Reproduction and Growth
NOEC = 0.30 mg a.i./L
LOEC = 0.54 mg a.i./L
MATC = 0.40 mg a.i./L

Survival
EC_{50} value = 1.2 mg a.i./L
95% confidence interval = 0.86 to 2.0 mg a.i./L

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: Upgrade to Core upon resolution of points 1,3, and 5 below and submission of missing raw data on reproduction.

C. Repairability: Repairable to Core

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.1300:

1.) The guidelines state to maintain cultures in 100% dilution water at test conditions (temperature, diet, background colors, and light intensity). However, the study reports that the culture solution temperature was maintained at 20 ± 2 °C, but test solution temperature ranged from 20 to 21 °C. In addition, there was a culture light intensity of 1200 to 1500 lux, but a test light intensity of 440 to 910 lux.

2.) The study reports that during culture daphnids were fed 2.0 mL of green algae and 0.5 mL of YCT suspension per test vessel once daily. During the definitive test, feeding consisted of 3.0 mL of algal suspension and 1.0 mL of YCT suspension per test vessel three times daily. This does not follow the guideline recommendation that during the test daphnids should be fed the same diet and at the same frequency as cultures.

3.) The study did not report water quality parameters for particulates, un-ionizable ammonia, residual chlorine, total organophosphorus pesticides 50 ng/L, total organochlorine pesticides plus PCBs or organic chlorine.

4.) The study did not report if test equipment and test chambers were cleaned before each use using good laboratory practices.

5.) The study did not report any information about aeration or if it was done before the addition of

the test substance.

6.) Dissolved oxygen concentrations in the study ranged from 91% to 109% saturation, however, the guidelines recommend between 60% and 105% saturation.

7.) The study reported that a range-finding test was conducted with exposure to nominal CL322,250 treatment levels of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.i./L and a control under flow-through conditions. The guidelines recommend exposure to a series of widely spaced concentrations of the test chemical (e.g., 1, 10, 100 mg/L), usually under static conditions.

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species •*Daphnia magna* •*D. pulex*	• *Daphnia magna*
Life Stage • First instar, ≤24 hours old.	• ≤ 24-hours old (p. 9)
Source • Daphnids should be cultured at the test facility and originate from same culture population.	• Springborn Smithers culture (p. 9, 13)
Culturing: • Source of initial stock and culturing techniques described. • Do not use daphnids if: • - Cultures contain ephippia. • - Adults in cultures do not produce young before day 12. • - More than 20% of the culture stock die during the 2 days preceding the test. • - Adults in the culture do not produce an average of at least three young per day over the 7-day period prior to test. • - Daphnids have been used in any portion of a previous test, either in a treatment or in a control.	• Yes. (p. 13) Adult daphnids use to produce offspring: • Did not contain ephippia (p. 13) • Produced offspring prior to being 12 days old (p. 13) • Survival 48 hours prior to test initiation was 100% (p. 13) • Produced an average of 12.5 offspring per female per day seven days prior to test initiation (p. 13) • Were not used in any portion of a previous test (p. 13)

Acclimation • Acclimate at least 48 hours prior to start of test. • Maintain in 100% dilution water at test conditions (temperature, diet, background colors, and light intensity). • Should be fed same food as used during definitive test	• Yes. (p. 13) • Culture solution temperature at 20 ± 2 °C, but test solution temperature ranged from 20 to 21 °C. Culture light intensity of 1200 to 1500 lux, but test light intensity of 440 to 910 lux. Test and culture photoperiod of 16 hours light and 8 hours darkness. (p. 13-15) • See below for details on feeding.
Feeding • During test, daphnids should be fed same diet and at same frequency as cultures. • Suggested rates: 5 to 7 mg/L of dilution water or test solution (automatic); 15 mg (dry weight)/L (manual).	• Culture feeding: 2.0 mL of green algae and 0.5 mL of YCT suspension per test vessel once daily. (p. 14) • Test feeding: 3.0 mL of algal suspension and 1.0 mL of YCT suspension per test vessel three times daily. (p.14)

B. Test System

Guideline Criteria	Reported Information
System • Static-renewal: dilution water completely replaced at least once every 3 days. • Flow-through: • - Calibrate system before each test. • - Check general operation at least twice during test. • - 24-hour flow through a test chamber should equal at least 5x volume of chamber. • - Flow rate should not vary by more than 10% from one chamber to another.	• Diluter system calibrated prior to test initiation and confirmed at test termination. (p. 16) • Function of the diluter system was monitored daily and a visual check of the operation was performed twice each day. (p. 16) • Test solutions were delivered to the exposure vessels at an approximate rate of 6 test vessel volumes per 24-hour period. (p. 17) • Flow-splitting accuracy was within 10% of the targeted delivery. (p. 16)

Dilution Water • Surface or ground water, reconstituted water, (deionized) water, or dechlorinated tap water acceptable. • Water quality parameters (maximum): • Particulates 20 mg/L • TOC 2 mg/L or COD 5 mg/L • Un-ionizable ammonia 20μg/L • Residual chlorine <3 µg/L • Total organophosphorus pesticides 50 ng/L • Total organochlorine pesticides plus PCBs (50 ng/L) or organic chlorine 25 ng/L • Water quality should be tested at least twice per year. • If diluent is groundwater or surface water, conductivity and TOC or COD should be measured.	• Fortified and filtered well water. (p. 14) • Water quality parameters were measured on each batch of fortified water prior to use. Fortified water was discarded if not used within 14 days of preparation. (p. 14) • TOC = 0.29 mg/L. Specific conductivity of 500 µmhos/cm. (p. 14)
Photoperiod • 16-hr light/8-hr dark	• Yes. (p. 15)
Test Chambers • 250-mL beakers or other suitable containers. • Loosely covered to reduce loss of test solution or dilution water due to evaporation and to minimize entry of dust or other particulates. • Test equipment and test chambers should be cleaned before each use using good laboratory practices. • For flow-through tests: daphnids can be in glass or stainless steel containers with stainless steel or nylon bottoms suspended in test chamber to ensure test solution flows regularly into and out of containers and daphnids are always submerged in at least 5 cm of test solution.	• 1.6-L glass battery jars. (p. 16) • Loosely covered with plastic. (p. 16) • Cleaning of test equipment and test chambers not reported. • Exposure solutions drained from each vessel through two 2-cm holes approximately 15 cm from the bottom of the glass jars which maintained the test solution volume of 1.4 L. Drain holes were covered with a Nitex 40-mesh screen. (p. 16)
Temperature • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations. • 20 ± 1°C	• Measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). (p. 18) • 20-21°C. (p. 23, 30)
Dissolved Oxygen • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations. • Between 60 and 105 percent saturation. • Aeration should be done before addition of test substance.	• Measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). (p. 18) • Dissolved oxygen = 8.1 to 9.9 mg/L (91% to 109%) (p. 23, 30) • Aeration was not discussed in the report.

pH • Measured at beginning of test and on days 7, 14, and 21 in at least 2 chambers of high, middle, and low, and control test concentrations.	• Measured in each test vessel at test initiation and weekly thereafter until test termination (day 21). (p. 18) • pH = 8.1 to 8.3. (p. 23, 30)
Solvents and Carriers • Concentration of carrier ≤0.1 mL/L. • Triethylene glycol and dimethyl formamide preferred solvents, but acetone or ethanol can be used if necessary.	• No use of solvent or carrier reported.

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Should be conducted to establish test solution concentrations in definitive test. • Exposure to a series of widely spaced concentrations of the test chemical (e.g., 1, 10, 100 mg/L), usually under static conditions. • Minimum of five daphnids should be exposed to each concentration of test substance. • Exposure period may be shortened if suitable data can be obtained in less time. • No replicates required and nominal concentrations of chemical acceptable.	• Range-finding test conducted. (p. 22) • Exposure to nominal CL322,250 treatment levels of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.i./L and a control under flow-through conditions. (p. 22) • Two replicate vessels (10 daphnids per vessel) were established for each concentration and four replicate vessels (10 daphnids per vessel) were established for the control. (p. 22)
Doses • Five or more concentration in a geometric series with a 1.5 to 2.0 progression (e.g., 2, 4, 8, 16, 32, and 64 mg/L).	• Nominal concentrations= control, 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.i./L (p. 23) • Mean measured concentrations = control, 0.11, 0.30, 0.54, 0.86, and 2.0 mg a.i./L. (p. 24)
Test Substance Concentration • At minimum, concentration of test chemical should be measured in each chamber before the test and on days 7, 14, and 21 of the test, and in at least one appropriate chamber whenever a malfunction is detected. • Concentrations of test substance in replicate test chambers should not vary more than ± 20%.	• Twice prior to initiation, samples were taken from both replicates of high, medium, low and control treatment levels and analyzed (p.19) • During study, water samples were removed from both replicates of each treatment level and the control and analyzed on test days 0, 7, 14, and 21(p. 19) • Concentrations did not vary more than ± 20%. (p. 31)

Controls • Controls should consist of same dilution water, conditions, and procedures, and daphnids. • Negative and/or solvent	• Yes. (p. 16, 17) • Negative control.
Replicates Per Dose • Equal number of daphnids in 2 or more replicates per dose (flow-through) • One daphnid each in 10 or more replicates per dose (static-renewal).	• 10 organisms per replicate vessel. (p. 17) • 2 replicates for each of 5 concentrations and the control. (p. 17)
Number of Organisms: • Minimum of 20 daphnids per concentration (flow-through). • Minimum of 10 daphnids per concentration (static-renewal). • Test organisms randomly or impartially placed in the test chambers. • Loading should not exceed 40 daphnids per liter of test solution in static-renewal system. • Loading in flow-through test varies depending on flow rate of test solution.	• Yes. (p. 17) • Daphnids were impartially added. (p. 17)
Duration of Test • 21 days	• Yes. (p. 9)
Observation of Daphnids • Daphnids in the test chambers observed on day 21 of the test. • Offspring should be counted and removed from the test chambers every 2 or 3 days. • Abnormal behavior or appearance reported.	• The number of immobilized offspring and adult daphnids and observations of abnormal behavior were recorded on days 0, 2, 4, 6, 7, 10, 13, 14, 17, 20 and 21. (p. 17) • Assessments of offspring released were determined beginning on day 8 and three times per week through day 21. (p. 17) • The total body length and dry weight of each surviving adult daphnid was measured on day 21. (p. 17-18)
Test Endpoints Measured • Number of daphnids immobilized (EC_{50} values and 95% C.I.) • Number of young per adult. • MATC determined for most sensitive endpoint.	• EC_{50} value = 1.2 mg a.i./L and 95% confidence interval = 0.86 to 2.0 mg a.i./L (p. 10) • Mean cumulative offspring per female. (p. 33) • MATC = 0.40 mg a.i./L (p. 10)
Growth • Determined by measuring total body length or dry weight (both preferred).	• The total body length and dry weight of each surviving adult daphnid was measured on day 21. (p. 18, 34)

Validity of Test Test is only valid if: • Less than 20% of the control should be immobilized, stressed, or diseased at the end of the study. • Each control daphnid should have produced at least 60 young after 21 days. • The controls should not produce any ephippia.	• Raw immobilization, stress, and disease data could not be found in the report. 95% survival of parental daphnids in control vessel after 21 days. (p. 32) • Mean cumulative number of offspring per control female was 154 after 21 days. (p. 24, 33) • The production of ephippia in control vessel was not discussed in the report.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes. (p. 3, 4)
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	• Yes. (cover page, p. 9)
Growth of the daphnids determined by total body length or body weight?	• Yes, weight and length. (p. 18, 34)
Source of test material, lot number, composition, known chemical and physical properties, and any carriers or other additives used and their concentrations reported?	• Source, batch number, and purity reported. (p. 12, 13) • Chemical/physical properties not reported.
Source of the dilution water, its chemical characteristics (e.g. conductivity, hardness, pH), and a description of any pretreatment reported?	• Yes. (p. 14-15)
Detailed information about the daphnids provided?	• Yes. (p. 13-14)
Description of the test chambers provided?	• Yes. (p. 16-17)
Concentration of the test substance in the test chambers at the designated times provided?	• Yes, at days 0, 7, 14, and 21. (p. 31)
Number and percentage of organisms that showed any adverse effect reported?	• Not provided in the study report, but the study states that these observations were recorded. (p. 17)
Cumulative adult and offspring immobilization values, progeny produced, the time to first brood, the number offspring per adult, and growth of surviving adults measured?	• Cumulative mean percent survival, mean cumulative number of offspring per female, and mean total body lengths and dry weights reported. (p. 32-34). • Time to first brood also reported. (p. 25)
All chemical analysis (of water quality) and test substance concentrations, including methods, method validation, and reagents reported?	• Yes. (Appendix 2)

Data records of the culture, acclimation, and test temperature provided?	• Data records of test temperature provided (p. 30) • Data records of the culture and acclimation period not provided.
Deviations from the test guideline provided and anything unusual about test (e.g., dilution failure, temperature fluctuations) reported?	• Protocol deviations provided. (p. 27)
MATC reported and statistical methods employed reported?	• Yes. (p. 10, 20-22)
Concentration-response curves utilizing average test substance concentration and adult immobilization data at 21 days provided?	• No
EC50 value based on adult immobilization calculated using the average measured concentration of the test substance?	• Yes. (p. 21)
Raw data included:	• Summary data only. (p. 30-34)
Statistical methods reported:	• Yes. (p. 20-21)

Dose Response

Test Group	Nominal Concentration (mg/L)	Mean Concentration (mg/L)	Mortality (%)	Mean No. Offspring Produced Over 21 Days	Mean Dry Weight at Day 21 (mg) (SD)	Mean Total Body Length at Day 21 (mm) (SD)
Control	0	0	5	154	1.01 (0.16)	4.65 (0.17)
1	0.13	0.11	0	148	0.91 (0.17)	4.59 (0.16)
2	0.25	0.30	5	160	0.97 (0.17)	4.67 (0.14)
3	0.50	0.54	0	135	0.84 (0.14)	4.45 (0.17)
4	1.0	0.86	5	104	0.70 (0.16)	4.22 (0.20)
5	2.0*	2.0*	100	0	NA	NA

*Treatment level excluded from statistical analysis due to statistically significant reduction in survival.

Statistical Results

Statistical Method: Survival, reproduction and growth data were analyzed to determine if there were any statistically significant treatment effects. Analyses were performed using the mean replicate organism response in each treatment group rather than individual response values. Survival, reproduction and grow data were checked for normality with the Shapiro-Wilks' Test and for homogeneity of variance using the Barlett's test. Survival and weight data were also analyzed for homogeneity of variance by Hartley's Test.

The NOECs and LOECs were calculated based on daphnid reproduction and growth. Statistical analysis for reproduction and total body length was performed using Williams' Test. Statistical analysis for dry weight was performed using Dunnett's Test. The MATC was calculated based on the geometric mean of the NOECs and LOECs. The 2.0 mg/L treatment level was excluded from statistical analysis of reproduction and growth data due to the statistically significant reduction in survival.

Based on daphnid survival, the 21-day EC_{50} value was calculated by binomial probability.

Results Synopsis:
Reproduction and Growth
NOEC = 0.30 mg a.i./L
LOEC = 0.54 mg a.i./L

MATC = 0.40 mg a.i./L

Survival
EC_{50} value = 1.2 mg a.i./L
95% confidence interval = 0.86 to 2.0 mg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:

NOEC/LOEC
Total body length and dry weight data were analyzed to determine if there were any statistically significant treatment effects. The data were first checked for normality using the Shapiro-Wilks test and for homogeneity of variances using Bartlett's test. The length data passed for both normality and homogeneity of variance. The dry weight data passed for normality but failed for homogeneity because of zero variance.

The NOECs and LOECs were then determined using Dunnett's test. For total body length, the test determined a significant difference in the 0.54 and 0.86 mg a.i./L treatment levels compared to the control. For dry weight, the test indicated a significant difference in mean dry weight in the 0.11, 0.54 and 0.86 mg a.i./L treatment levels compared to the control. The study reported the same findings, however, the observed reduction in dry weight at the 0.11 mg a.i./L treatment level was not considered to be biologically relevant due to the lack of a similar reduction at the next highest treatment level. Therefore, the study's results of a NOEC of 0.30 mg a.i./L and a LOEC of 0.54 mg a.i./L for growth were verified. The 2.0 mg/L treatment level was excluded from statistical analysis of reproduction and growth data due to the statistically significant reduction in survival.

Reproduction results could not be verified because raw data were not provided in the report.

MATC
The MATC was calculated by taking the geometric mean of the NOEC and LOEC. The MATC was determined to be 0.40 mg a.i./L and matched the result stated in the study report.

EC_{50} Value
The EC_{50} results for survival could not be verified because raw data were not provided in the study report.

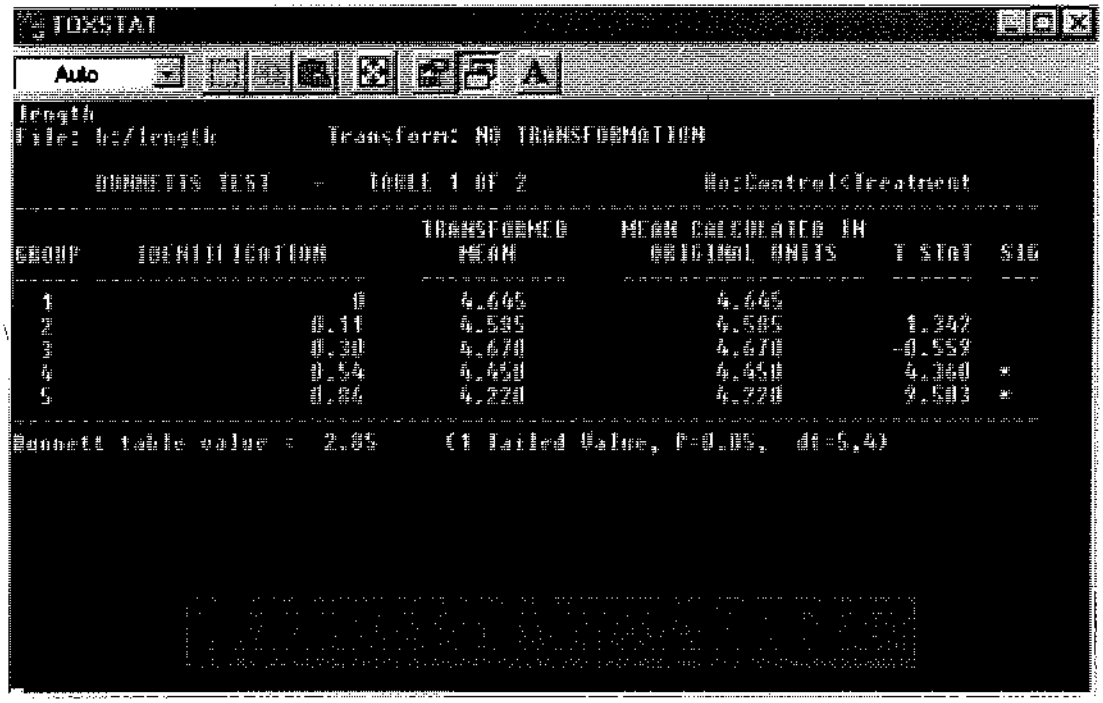
Results Verification Synopsis:
Growth
NOEC = 0.30 mg a.i./L
LOEC = 0.54 mg a.i./L

MATC = 0.40 mg a.i./L

14. REVIEWER'S COMMENTS:

- Guideline deviations are noted in Section 9.
- Reproduction NOEC and LOEC results could not be verified because raw data were not provided in the report.
- The EC_{50} results for survival could not be verified because raw data were not provided in the study report.

Daphnia Total Body Length-NOEC/LOEC



TOXSTAT

Auto

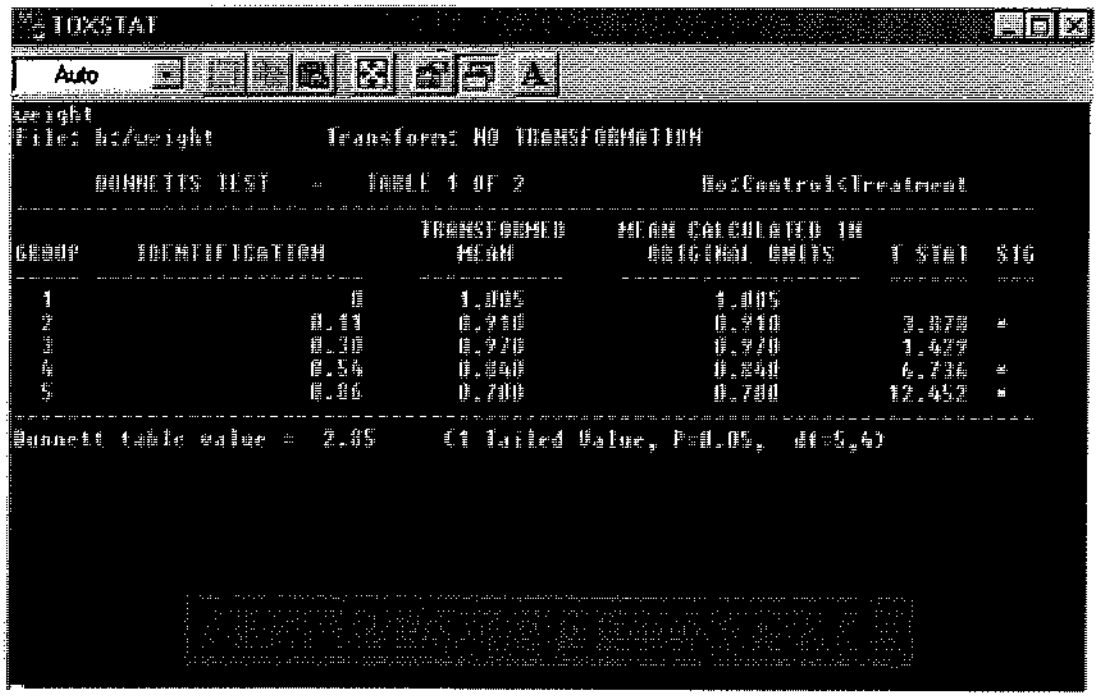
Length
File: A:/length Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	4.645	4.645		
2	0.11	4.585	4.585	1.342	
3	0.30	4.670	4.670	-0.559	
4	0.54	4.450	4.450	4.360	*
5	0.86	4.220	4.220	9.503	*

Dunnett table value = 2.05 (1 Tailed Value, P=0.05, df=5,40)

Daphnia Dry Weight-NOEC/LOEC



TOXSTAT

Auto

Weight
File: A:/weight Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	1.005	1.005		
2	0.11	0.910	0.910	3.879	*
3	0.30	0.970	0.970	1.427	
4	0.54	0.840	0.840	6.736	*
5	0.86	0.700	0.700	12.452	*

Dunnett table value = 2.05 (1 Tailed Value, P=0.05, df=5,40)

DATA EVALUATION RECORD
MYSID CHRONIC TOXICITY TEST
GUIDELINE OPPTS 850.1350

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)- (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** CL322,250 **Purity:** 88.2%

3. **CITATION**

Author:	Mark A. Calarella
Title:	CL322,250-Life-Cycle Toxicity Test with Mysids (*Americamysis bahia)*
Study Completion Date:	July 11, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1075
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No.: 13751.6153 Sponsor Protocol/Project No.: AGR 927
MRID No.:	465960-12

4. **REVIEWED BY:**

   Signature: **Date: 10/12/06**
   David Bays, RASSB, AD (7510C)

5. **APPROVED BY:**

   Signature: **Date: 10/12/06**
   Norm Cook, Branch Chief, RSSAB, AD

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Americamysis bahia*
Age of Test Organism:	≤24 hours old
Definitive Test Duration:	28-days; April 22 to May 20, 2005
Study Method:	Flow through
Type of Concentrations:	Nominal and mean measured

7. **CONCLUSIONS**

Results Synopsis:

LC_{50}: > 620 ug ai/L
LOEC: 160 ug ai/L
NOEC: 82 ug ai/L
MATC: 110 ug ai/L

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Scientifically Acceptable Study

C. Repairable: Registrant adequately resolved the guideline deviations. (See MRIDs 46917901, 46917903 and comment below)

9. GUIDELINE DEVIATIONS:

Guideline deviations and one review oversight were addressed in Bold:

- The study did not provide information on taxonomic verification of mysid species and notation of abnormalities at the time of receipt. **(The information can be verified in culture records maintained at Springborn Smithers Labs. Taxonomic verification of the species tested is provided by the supplier)**
- Cleaning procedures prior to test initiation of materials/instrumentation not provided. **(As is standard operating procedures at Springborn Smithers labs, all exposure systems (diluters, test chambers, etc) are thoroughly cleaned prior to each use)**
- Information on separation/location of offspring once born not provided. Guidelines state that as offspring are produced, young should be counted and separated into retention chambers with test concentration similar to that of original chambers. **(Offspring were not retained for observations in this study)**
- Mean data on survival, body length, and dry weight of offspring prior to or at termination not provided. Guidelines state that if available before test termination, observations on mortality, number of males and females, and male and female body length should be recorded for offspring. **(The data was not collected during this study)** These are
- Aquaria heaters maintained system temperatures at 26±2°C; not guideline specified 25±2°C. **(Guideline states that the test temperature should be maintained at 25 +/- 2C; however since the protocol was written to meet the requirements of the ASTM guideline as well (27 +/- 2C), the temperature range was modified slightly to cover both ranges)**
- The study did not provide concentration-response curves fitted to the cumulative number of adult dead for days 7, 14, 21, and 28 or the statistical test of goodness-of-fit performed and results reported. A survival bar graph (survival in %=100-dead in %) was provided, but does not fulfill the guideline requirements. **(No concentration-dependent response was observed for survival, nor were any statically-significant effects noted)**
- A review oversight occurred when only 10% of females in the control group produced young and less than 3 young were produced per female. According to the guideline, at least 75% of the females in the control group should produce young and more than 3 young should be produced per female for test to be acceptable. **(Reproductive information was collected for each of the ten pairs.** 100% of the females

in each replicate test vessel of the control produced young, exceeding the 75% minimum. The average number of offspring per female was 16.0 and 14.3 for the A and B replicates, respectively, exceeding the guideline requirements of 3 per offspring per female)

- (The raw data to verify reproduction and growth endpoints was provided by the testing Lab in the form of a supplemental report and was acceptable. MRID# - 469179-03)

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • Mysids (*Mysidopsis bahia*; now *Americamysis*)	• Mysids (*Americamysis bahia*)
Life Stage/Size • Juvenile mysids, #24-hours old used to start test. • Mysids used in a particular test should be of similar age and be of normal size and appearance for their age. • Should not exhibit abnormal behavior.	• Juvenile mysids, #24-hours old used • Information on abnormalities not provided (p. 13) but culture records are available and maintained by the supplier
Acquisition • Mysids should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. • Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. • Taxonomic verification should be obtained from the commercial supplier by experienced laboratory personnel or by an outside expert.	• Obtained from SSL laboratories (Lot No. 05A64) • Cultures were purchased commercially. • Information on taxonomic verification not provided, but is maintained by the supplier
Acclimation • Within a 24-h period, changes in water temperature should not exceed 1EC, while salinity changes should not exceed 5 percent. • During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.	• Heaters were used to maintain the temperature at 26°C. • Salinity varied by one ppt (20-21 ppt). • Mysids were maintained at similar conditions during testing and culture. (p. 14 and 16-17)

B. Test System

Guideline Criteria	Reported Information
<u>Test Chamber and Delivery System</u> • Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions. • Proportional diluters, metering pumps or other suitable system should be used to deliver test substance to test chambers • System should be calibrated before each test to determine flow rate and concentration of test substance in each chamber. • Check operation of delivery system at least twice daily during a test. • 24-hour flow rate through a chamber should be equal to at least 5x the volume of the chamber. • The flow rates should not vary more than 10 percent among chambers or across time. • Test substance delivery systems and test chambers should be cleaned before each use following standard laboratory practices.	• Chambers were covered with 350 µm mesh (Nitex® screen collar) that was attached with silicone. (p. 16) • Modified intermittent-flow proportional diluter used to deliver test substance (p. 15) • Proper operation of the system was calibrated and allowed to reach equilibrium prior to test initiation. (p. 16) • Visual checks were performed twice daily (p. 16) • The diluter provided approximately 7.4 aquarium volume additions per day. (p. 16) • The flow splitting accuracy was within 5% of the target delivery; however, flow splitting data was not recorded for the first concentration splitter (660 µg a.i./L) and third concentration (170 µg a.i./L). Analytical results and DO concentrations of replicates A and B within the test concentrations demonstrated that the splitters functioned properly. (p. 16 & 26) • Cleaning procedures prior to test initiation were conducted per Springborn SOP.
<u>Temperature</u> • Measured weekly in each chamber. • 25 ± 2EC	• Temperature measured daily in each replicate of each treatment level and control solutions (p. 19) • Temperature was monitored continuously in one control vessel. • Temperature ranged from 25 to 27°C during daily measurements and from 26 to 28°C from continuous measurements (p. 23)
<u>Salinity</u> • Measured weekly in each chamber. • Salinity of 20 ± 3 parts per thousand.	• Measured daily in each replicate of each treatment level. (p. 19 & 23) • Salinity ranged from 19-22 ppt; however, salinity measurements were not conducted in two treatments (660 and 330 µg a.i./L) of a replicate (B) at test termination. (p. 23 & 26)
<u>Dissolved Oxygen</u> • Measured weekly in each chamber. • Between 60 and 105 percent of saturation. • Aeration can be used to achieve this level; but should be done before addition of test substance	• Measured daily in each replicate of each treatment level. (p. 19) • Ranged between 92 and 100% throughout test period. (p. 23)

Photoperiod • Photoperiod of 14 hours light and 10 hours dark, with a 15 to 30 min transition period.	• Photoperiod of 16 hours of light followed by 8 hours of darkness with a 15 to 30 minute transition. (p. 16 & 45)
pH • Measured weekly in each chamber.	• Measured daily in each replicate of each treatment level. (p. 19)
Concentration of Test Substance • Determine concentration of test substance in test solutions at the beginning of the test and on days 7, 14, 21, and 28. • Measure concentration in at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. • Measured concentration should not vary more than 20 percent among replicate chambers.	• Concentrations measured on day 0 (initiation), 7, 14, 21, and 28 (termination). Mean measured concentrations ranged from 93 to 110%. (p.24 & 30) • Malfunctions were not noted and QC samples were measured for appropriate concentrations and demonstrated precision and quality control of the analytical method. (p. 30 & 24) • Concentrations between replicate chambers (A and B) not noted. (p. 24)
Feeding • Mysids should be fed during testing. • A recommended food is live *Artemia spp.* nauplii (48 hours old).	• Fed throughout study, twice daily. • Live brine shrimp (*Artemia salina*) nauplii • Prior to pairing one of the two feedings was with Selco® (a substance high in fatty acid) and after pairing the enriched shrimp was fed every other day. (p. 18)
Dilution Water • Natural seawater or artificial seawater is acceptable as dilution water if mysids will survive and successfully reproduce in it for the duration of the holding, acclimating, and testing periods without showing signs of stress. • Mysids should be cultured and tested in dilution water from the same origin. • Natural seawater should be filtered through a filter with a pore size of >20 μm prior to use in a test. • Artificial seawater can be prepared by adding commercially available formulations or specific amounts of reagent-grade chemicals to deionized water (conductivity <0.1 mS/m at 12°C) • If artificial seawater prepared from ground or surface water, conductivity and total organic carbon should be measured.	• Artificial seawater was synthesized on the beginning of each test day in a batch. No signs of toxicity noted. (p. 14) • Different water was used in the culture and testing phase, the two waters had similar chemical properties. (p. 14) • Commercially prepared salt formula was added to laboratory well water. Study protocol states that synthetic seawater will have a pH range of 8.0 to 8.5. At termination (day 28), one batch of synthetic seawater was characterized as having a pH of 7.9. (p. 14) • TOC concentration was analyzed and found to 0.84-0.46 mg/L. (p. 14)

Carriers/Solvents • If required, should be commonly used carriers and should not possess a synergistic or antagonistic effect on toxicity of test substance • Concentration of solvent should not exceed 0.1 ml/L	• Solvents and carriers not utilized (p. 15)

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Mysids should be exposed to a series of widely spaced concentrations of test substance (e.g., 1, 10, 100 mg/L), usually under static conditions. • Minimum of 10 mysids exposed to each concentration for a period of time sufficient to estimate appropriate chronic test concentrations. • No replicates required • Nominal concentrations acceptable	• Nominal concentrations of 21, 41, 83, 170 and 330 µg a.i./L and a dilution water control administered through flow through conditions were tested (duration = 22 day). • There were 30 mysids per replicate, where each treatment level had one replicate. • Nominal definitive concentrations were based on the results of this test which included: offspring per female per day, total length, and dry weight of mysids. (p. 23)
Doses • At least 5 test concentrations should be used. • Geometric series with ratio between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32 and 64 mg/L).	• 5 doses • 41, 83, 170, 330, 660 µg a.i./L, and a diluted water control. Ratios were approximately 2. (p. 23)
Controls • Every test should include controls consisting of the same dilution water, conditions, and procedures, and mysids from the same population or culture container, except that none of the test chemical is added.	• Yes (p. 17)
Replicates Per Dose • Should be separated into replicate groups of no more than 8 individuals when most of mysids reach sexual maturity (usually 10-14 days after beginning of test)	• Mature male and female mysids were paired into one of ten pairing jars within two retention chambers (24 retention chambers in total; 4 chambers per treatment level). Pairing occurred after 14 days. (p. 17)
Number and Placement of Organisms: • Test is started by randomly introducing acclimated mysids into retention chambers within the test and the control chambers. • Minimum of 40 mysids per concentration.	• Organisms were randomly selected to obtain 60 organisms per treatment level and control. (p. 17)
Duration of Test • 28 days	• 28 days

Measurements/Observations • Number of dead mysids, cumulative young per female, and body length of males and females recorded • Number of dead mysids recorded on days 7,14,21,and 28 • Number of male and female mysids should be recorded when discernible (around 10-12 days in controls; may be longer in treatments) • Any abnormal behavior should be recorded • As offspring are produced, young should be counted and separated into retention chambers with test concentration similar to that of original chambers • If available before test termination, observations on mortality, number of males and females, and male and female body length should be recorded for offspring. • >75% of the females in the control group must produce young or the average number of young produced per female in the controls must be >3 per day for test to be acceptable	• Daily survival, cumulative young per female, body length, and dry weight of both males and females. (p. 24 & 29-30 & Appendix 3) • Number survived and dead recorded daily (p. 18) • Abnormal appearance and behavior was recorded. (p. 18) • Separation of offspring into retention chambers was not noted. • Summary data not provided for offspring prior to or at test termination. Offspring were not retained for observation (pg 13 of MRID 465960-12). • 100% of females in each replicate of the controls produced young (pg 31, Table 3 of initial study report), w/ 14.3 and 16.0 young produced mas >3 per day.

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes, as well as Statement of No Data Confidentiality Claim.
The nature of the test, laboratory, name of the investigator, test substance, and dates of test reported?	Yes (p. 5 and 9-10)
Source of the dilution water, its chemical characteristics (e.g. salinity, pH, etc.), and a description of any pretreatment provided?	Yes (p. 14)
Detailed information about the test organisms, including the scientific name and method of verification, average length, age, source, history, observed diseases, treatments, acclimation procedures, and food used provided?	Yes (p. 13-14)

A description of the test chambers, the depth and volume of solution in the chamber, the way the test was begun (e.g., conditioning, test substance additions, etc.), the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system, and the flow rate expressed as volume additions per 24 hours provided?	Yes (p. 15-17)
The measured concentration of test substance in test chambers at times designated?	Yes (p. 30)
First time (day) that sexual characteristics can be observed in controls and in each test substance concentration reported?	Yes, day 14 (p. 17)
Length of time for appearance of first brood for each concentration reported?	Yes, day 15 (p. 24)
Means (average of replicates) and respective 95 percent CIs for: **-- body length of males and females at first observation day (depending on time of sexual maturation) and on day 28?** **– cumulative number of young produced per female on day 28?** **– cumulative number of dead adults on day 7, 14, 21, and 28?**	Means were provided for all data, but not 95 percent CI. --Body lengths were provided on day 28, only (p. 32) --Yes, (p. 31) --Yes, (p. 82-87)
If available, effects on G2 mysids (number of males and females, body length of males and females, and cumulative mortality reported?	Not available
MATC determined for the most sensitive test criteria measured (cumulative mortality of adult mysids, number of young per female, or body lengths of adult males and females)?	MATC determined to be 110 µg a.i./L and was based on statistical analysis of mysid reproduction. (p.10)
Concentration-response curves fitted to the cumulative number of adult dead for days 7, 14, 21, and 28? Statistical test of goodness-of-fit performed and results reported?	Not reported
LC_{50} value based on number of dead adults with corresponding 95% CIs for days 7, 14, 21, and 28 determined? Calculations made using average measured concentrations of test substance?	The LC_{50} for day 21 was reported to be empirically estimated to be greater than 620 µg a.i./L, the highest mean measured concentration measured, since no concentration tested resulted in greater than or equal to 50% mortality.
Methods and data records of all chemical analyses of water quality and test substance concentrations (including method validations and reagent blanks) reported?	Yes, appendix 2 (p. 55)
Data records of holding, acclimation, test temperature, and salinity reported?	Yes

Dose Response

First Generation Survival and Reproductive Success (Offspring/Female/Reproductive Day) at Termination on Day 28:

Nominal Concentration (µg a.i./L)	Mean Measured Concentration (µg a.i./L)	Replicate	Percent Survival[a]	Percent Survival[b]	Number of Females Producing Young	Number of Offspring per Female	Reproductive Success
Control	Control	A	77	80	10	16.0	1.14
		B	77	80	10	14.3	1.02
		Mean	77	80			1.08
41	44	A	70	60	10	12.7	0.95
		B	90	95	10	13.1	0.96
		Mean	80	78			0.96
83	82	A	93	95	9	12.1	0.87
		B	73	95	9	14.4	1.05
		Mean	83	95			0.96
170	160	A	87	85	9	10.1	0.74
		B	90	95	10	10.6	0.76
		Mean	88	90			0.75[c]
330	310	A	80	75	7	12.4	0.76
		B	73	70	9	8.0	0.53
		Mean	77	73			0.64[c]
660	620	A	77	80	10	9.7	0.73
		B	63	55	10	13.0	1.06
		Mean	70	68			0.89[c]

a Study Report states that the values presented were rounded to two significant figures.

b The Study Report stated that daily survival data were presented in Appendix 3 of the report. Using that raw data, Versar calculated percent survival, but was unable to verify the values reported in the Study Report. It is unclear as to what is causing the discrepancy.

c The Study Report stated that these values were statistically different compared to control, based on Williams' Test.

Average Total Body Length at Termination on Day 28:

Nominal Concentration (µg a.i./L)	Mean Measured Concentration (µg a.i./L)	Replicate	Mean Total Body Length (mm)	
			Males	Females
Control	Control	A	6.1	6.4
		B	6.6	6.8
		Mean	6.4	6.6
41	44	A	7.3	7.0
		B	7.1	7.1
		Mean	7.2	7.1
83	82	A	7.1	6.9
		B	6.7	6.2
		Mean	6.9	6.6
170	160	A	6.5	6.8
		B	7.0	6.9
		Mean	6.8	6.9

			Mean Total Body Length (mm)	
330	310	A	7.1	6.9
		B	6.9	6.9
		Mean	7.0	6.9
660	620	A	6.8	6.9
		B	6.8	7.0
		Mean	6.8	6.9

a Study Report states that the values presented were rounded to two significant figures, however, statistical analysis was performed using unrounded values.

Dry Body Weight at Termination on Day 28:

Nominal Concentration (µg a.i./L)	Mean Measured Concentration (µg a.i./L)	Replicate	Mean Dry Body Weight (mg)	
			Males	Females
Control	Control	A	0.79	1.01
		B	0.80	1.18
		Mean	0.80	1.09
41	44	A	0.93	1.15
		B	0.93	1.19
		Mean	0.93	1.17
83	82	A	0.88	1.02
		B	0.78	1.09
		Mean	0.83	1.05
170	160	A	0.82	1.07
		B	0.87	1.17
		Mean	0.85	1.12
330	310	A	0.87	0.97
		B	0.86	1.16
		Mean	0.86	1.07
660	620	A	0.93	0.95
		B	1.01	1.05
		Mean	0.96	1.00

Statistical Results

Statistical Method:
The endpoints examined in the study included day 28 survival, growth in terms of average dry body weight and average total length) and reproduction. The MATC, LOEC and NOEC were obtained and significant differences in the percent survival were determined. Mysid data on survival, reproduction, and growth was tested for normality using Shapiro-Wilk's Test (Weber, et al., 1989), homogeneity using Barlett's Test (Horning and Weber, 1985) or Cochran's test, and were tested for statistical differences from the control using Williams' Test (Williams, 1971, 1972).

The LC_{50} was empirically estimated to be greater than the highest concentration tested since no concentration tested results in a greater than 50% mortality and no statistical analyses were performed.

Results Synopsis: ~~**INVALID**~~ CORE MCok
LC_{50}: >620 µg a.i./**L** (day 21)
LOEC: 160 µg a.i./**L**
NOEC: 82 µg a.i./L
MATC: 110 µg a.i./l.

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:
Initial (before test termination) raw data on body length, dry weight, and reproduction were not provided. The data are requested, but not required to be reported. The study report found mysid reproduction to be the most sensitive performance criteria. Raw data on offspring per female on each test date were provided in MRID 46917903.

MATC
The MATC is calculated to be the geometric mean of the LOEC and NOEC. Using the report's finding (LOEC = 160 µg a.i./L and NOEC= 82 µg a.i./**L**), the calculated MATC as 114 mg a.i./**L**. Rounded to two significant figures, this value (110 mg a.i./L) agrees with that reported in the Study Report.

LC_{50} Value
The Toxanal program was used to calculate LC_{50} values. However, the program found that none of the mortality rates were greater than 50% of the control group indicating that the LC_{50} value for day 28 is greater than the highest concentration tested (620 µg a.i./L).

Results Verification Synopsis:

MATC:	114 mg a.i./L (rounded with two significant figures: 110 mg a.i./L)
LC_{50}:	>620 µg a.i./L

14. REVIEWER'S COMMENTS:

No additional comments.

DP Barcode: 321452 MRID No: 465960-12

DATA EVALUATION RECORD
MYSID CHRONIC TOXICITY TEST
GUIDELINE OPPTS 850.1350

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)- (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** CL322,250 **Purity:** 88.2%

3. **CITATION**

Author:	Mark A. Cafarella
Title:	CL322,250-Life-Cycle Toxicity Test with Mysids (*Americamysis bahia)*
Study Completion Date:	July 11, 2005
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1075
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No.: I3751.6153 Sponsor Protocol/Project No.: AGR 927
MRID No.:	465960-12

4. **REVIEWED BY:**

   Signature: Agronomist DACO/IBD **Date:** 4/11/06

5. **APPROVED BY:**

   Signature: **Date:** 4/17/06

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Americamysis bahia*
Age of Test Organism:	≤24 hours old
Definitive Test Duration:	28-days; April 22 to May 20, 2005
Study Method:	Flow through
Type of Concentrations:	Nominal and mean measured

7. **CONCLUSIONS**

Results Synopsis:

LC_{50}:
LOEC:
NOEC:
MATC:

The LOEC and NOEC could not be verified due to the lack of raw data on individual reproduction of females per treatment day.

8. ADEQUACY OF THE STUDY

A. **Classification: Invalid**

B. **Rationale: Very low reproductive count in the controls (only 10% in test vs a minimum of 75% required). Test lacks enough raw data to perform statistical analysis. See other deviations below (9.).**

C. **Repairable?: No**

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1350:

- The study did not provide information on taxonomic verification of mysid species and notation of abnormalities at the time of receipt.
- Cleaning procedures prior to test initiation of materials/instrumentation not provided.
- Information on separation/location of offspring once born not provided. Guidelines state that as offspring are produced, young should be counted and separated into retention chambers with test concentration similar to that of original chambers.
- Mean data on survival, body length, and dry weight of offspring prior to or at termination not provided. Guidelines state that if available before test termination, observations on mortality, number of males and females, and male and female body length should be recorded for offspring.
- Aquaria heaters maintained system temperatures at 26±2°C; not guideline specified 25±2°C.
- The study did not provide concentration-response curves fitted to the cumulative number of adult dead for days 7, 14, 21, and 28 or the statistical test of goodness-of-fit performed and results reported. A survival bar graph (survival in %=100-dead in %) was provided, but does not fulfill the guideline requirements.
- Only 10% of females in the control group produced young and less than 3 young were produced per female. According to the guideline, at least 75% of the females in the control group should produce young and more than 3 young should be produced per female for test to be acceptable.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information

Species • Mysids (*Mysidopsis bahia*; now *Americamysis*)	• Mysids (*Americamysis bahia)*
Life Stage/Size • Juvenile mysids, ≤24-hours old used to start test. • Mysids used in a particular test should be of similar age and be of normal size and appearance for their age. • Should not exhibit abnormal behavior.	• Juvenile mysids, ≤24-hours old used • Information on abnormalities not provided (p. 13)
Acquisition • Mysids should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. • Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. • Taxonomic verification should be obtained from the commercial supplier by experienced laboratory personnel or by an outside expert.	• Obtained from SSL laboratories (Lot No. 05A64) • Cultures were purchased commercially. • Information on taxonomic verification not provided. (p. 13)
Acclimation • Within a 24–h period, changes in water temperature should not exceed 1 °C, while salinity changes should not exceed 5 percent. • During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.	• Heaters were used to maintain the temperature at 26°C. • Salinity varied by one ppt (20-21 ppt). • Mysids were maintained at similar conditions during testing and culture. (p. 14 and 16-17)

B. Test System

Guideline Criteria	Reported Information
Test Chamber and Delivery System • Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions. • Proportional diluters, metering pumps or other suitable system should be used to deliver test substance to test chambers • System should be calibrated before each test to determine flow rate and concentration of test substance in each chamber. • Check operation of delivery system at least twice daily during a test. • 24-hour flow rate through a chamber should be equal to at least 5x the volume of the chamber. • The flow rates should not vary more than 10 percent among chambers or across time. • Test substance delivery systems and test chambers should be cleaned before each use following standard laboratory practices.	• Chambers were covered with 350 µm mesh (Nitex® screen collar) that was attached with silicone. (p. 16) • Modified intermittent-flow proportional diluter used to deliver test substance (p. 15) • Proper operation of the system was calibrated and allowed to reach equilibrium prior to test initiation. (p. 16) • Visual checks were performed twice daily (p. 16) • The diluter provided approximately 7.4 aquarium volume additions per day. (p. 16) • The flow splitting accuracy was within 5% of the target delivery; however, flow splitting data was not recorded for the first concentration splitter (660 µg a.i./L) and third concentration (170 µg a.i./L). Analytical results and DO concentrations of replicates A and B within the test concentrations demonstrated that the splitters functioned properly. (p. 16 & 26) • Cleaning procedures prior to test initiation were not provided.
Temperature • Measured weekly in each chamber. • 25 ± 2°C	• Temperature measured daily in each replicate of each treatment level and control solutions (p. 19) • Temperature was monitored continuously in one control vessel. • Temperature ranged from 25 to 27°C during daily measurements and from 26 to 28°C from continuous measurements (p. 23)
Salinity • Measured weekly in each chamber. • Salinity of 20 ± 3 parts per thousand.	• Measured daily in each replicate of each treatment level. (p. 19 & 23) • Salinity ranged from 19-22 ppt; however, salinity measurements were not conducted in two treatments (660 and 330 µg a.i./L) of a replicate (B) at test termination. (p. 23 & 26)
Dissolved Oxygen • Measured weekly in each chamber. • Between 60 and 105 percent of saturation. • Aeration can be used to achieve this level; but should be done before addition of test substance	• Measured daily in each replicate of each treatment level. (p. 19) • Ranged between 92 and 100% throughout test period. (p. 23)

Photoperiod • Photoperiod of 14 hours light and 10 hours dark, with a 15 to 30 min transition period.	• Photoperiod of 16 hours of light followed by 8 hours of darkness with a 15 to 30 minute transition. (p. 16 & 45)
pH • Measured weekly in each chamber.	• Measured daily in each replicate of each treatment level. (p. 19)
Concentration of Test Substance • Determine concentration of test substance in test solutions at the beginning of the test and on days 7, 14, 21, and 28. • Measure concentration in at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. • Measured concentration should not vary more than 20 percent among replicate chambers.	• Concentrations measured on day 0 (initiation), 7, 14, 21, and 28 (termination). Mean measured concentrations ranged from 93 to 110%. (p.24 & 30) • Malfunctions were not noted and QC samples were measured for appropriate concentrations and demonstrated precision and quality control of the analytical method. (p. 30 & 24) • Concentrations between replicate chambers (A and B) not noted. (p. 24)
Feeding • Mysids should be fed during testing. • A recommended food is live *Artemia spp.* nauplii (48 hours old).	• Fed throughout study, twice daily. • Live brine shrimp (*Artemia salina*) nauplii • Prior to pairing one of the two feedings was with Selco® (a substance high in fatty acid) and after pairing the enriched shrimp was fed every other day. (p. 18)
Dilution Water • Natural seawater or artificial seawater is acceptable as dilution water if mysids will survive and successfully reproduce in it for the duration of the holding, acclimating, and testing periods without showing signs of stress. • Mysids should be cultured and tested in dilution water from the same origin. • Natural seawater should be filtered through a filter with a pore size of >20 μm prior to use in a test. • Artificial seawater can be prepared by adding commercially available formulations or specific amounts of reagent-grade chemicals to deionized water (conductivity <0.1 mS/m at 12°C) • If artificial seawater prepared from ground or surface water, conductivity and total organic carbon should be measured.	• Artificial seawater was synthesized on the beginning of each test day in a batch. No signs of toxicity noted. (p. 14) • Different water was used in the culture and testing phase, the two waters had similar chemical properties. (p. 14) • Commercially prepared salt formula was added to laboratory well water. Study protocol states that synthetic seawater will have a pH range of 8.0 to 8.5. At termination (day 28), one batch of synthetic seawater was characterized as having a pH of 7.9. (p. 14) • TOC concentration was analyzed and found to 0.84-0.46 mg/L. (p. 14)

Carriers/Solvents • If required, should be commonly used carriers and should not possess a synergistic or antagonistic effect on toxicity of test substance • Concentration of solvent should not exceed 0.1 mL/L	• Solvents and carriers not utilized (p. 15)

C. **Test Design**

Guideline Criteria	Reported Information
Range-Finding Test • Mysids should be exposed to a series of widely spaced concentrations of test substance (e.g., 1, 10, 100 mg/L), usually under static conditions. • Minimum of 10 mysids exposed to each concentration for a period of time sufficient to estimate appropriate chronic test concentrations. • No replicates required • Nominal concentrations acceptable	• Nominal concentrations of 21, 41, 83, 170 and 330 µg a.i./L and a dilution water control administered through flow through conditions were tested (duration = 22 day). • There were 30 mysids per replicate, where each treatment level had one replicate. • Nominal definitive concentrations were based on the results of this test which included: offspring per female per day, total length, and dry weight of mysids. (p. 23)
Doses • At least 5 test concentrations should be used. • Geometric series with ratio between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32 and 64 mg/L).	• 5 doses • 41, 83, 170, 330, 660 µg a.i./L, and a diluted water control. Ratios were approximately 2. (p. 23)
Controls • Every test should include controls consisting of the same dilution water, conditions, and procedures, and mysids from the same population or culture container, except that none of the test chemical is added.	• Yes (p. 17)
Replicates Per Dose • Should be separated into replicate groups of no more than 8 individuals when most of mysids reach sexual maturity (usually 10-14 days after beginning of test)	• Mature male and female mysids were paired into one of ten pairing jars within two retention chambers (24 retention chambers in total; 4 chambers per treatment level). Pairing occurred after 14 days. (p. 17)
Number and Placement of Organisms: • Test is started by randomly introducing acclimated mysids into retention chambers within the test and the control chambers. • Minimum of 40 mysids per concentration.	• Organisms were randomly selected to obtain 60 organisms per treatment level and control. (p. 17)
Duration of Test • 28 days	• 28 days

Measurements/Observations • Number of dead mysids, cumulative young per female, and body length of males and females recorded • Number of dead mysids recorded on days 7,14,21,and 28 • Number of male and female mysids should be recorded when discernible (around 10-12 days in controls; may be longer in treatments) • Any abnormal behavior should be recorded • As offspring are produced, young should be counted and separated into retention chambers with test concentration similar to that of original chambers • If available before test termination, observations on mortality, number of males and females, and male and female body length should be recorded for offspring. • >75% of the females in the control group must produce young or the average number of young produced per female in the controls must be >3 per day for test to be acceptable	• Daily survival, cumulative young per female, body length, and dry weight of both males and females. (p. 24 & 29-30 & Appendix 3) • Number survived and dead recorded daily (p. 18) • Abnormal appearance and behavior was recorded. (p. 18) • Separation of offspring into retention chambers was not noted. • Summary data not provided for offspring prior to or at test termination. • 10% of females in the control group produced young and less than 3 young were produced per female. (p. 31)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes, as well as Statement of No Data Confidentiality Claim.
The nature of the test, laboratory, name of the investigator, test substance, and dates of test reported?	Yes (p. 5 and 9-10)
Source of the dilution water, its chemical characteristics (e.g. salinity, pH, etc.), and a description of any pretreatment provided?	Yes (p. 14)
Detailed information about the test organisms, including the scientific name and method of verification, average length, age, source, history, observed diseases, treatments, acclimation procedures, and food used provided?	Yes (p. 13-14)

A description of the test chambers, the depth and volume of solution in the chamber, the way the test was begun (e.g., conditioning, test substance additions, etc.), the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system, and the flow rate expressed as volume additions per 24 hours provided?	Yes (p. 15-17)
The measured concentration of test substance in test chambers at times designated?	Yes (p. 30)
First time (day) that sexual characteristics can be observed in controls and in each test substance concentration reported?	Yes, day 14 (p. 17)
Length of time for appearance of first brood for each concentration reported?	Yes, day 15 (p. 24)
Means (average of replicates) and respective 95 percent CIs for: **-- body length of males and females at first observation day (depending on time of sexual maturation) and on day 28?** **– cumulative number of young produced per female on day 28?** **– cumulative number of dead adults on day 7, 14, 21, and 28?**	Means were provided for all data, but not 95 percent CI. --Body lengths were provided on day 28, only (p. 32) --Yes, (p. 31) --Yes, (p. 82-87)
If available, effects on G2 mysids (number of males and females, body length of males and females, and cumulative mortality reported?	Not available
MATC determined for the most sensitive test criteria measured (cumulative mortality of adult mysids, number of young per female, or body lengths of adult males and females)?	MATC determined to be 110 µg a.i./L and was based on statistical analysis of mysid reproduction. (p.10)
Concentration-response curves fitted to the cumulative number of adult dead for days 7, 14, 21, and 28? Statistical test of goodness-of-fit performed and results reported?	Not reported
LC_{50} value based on number of dead adults with corresponding 95% CIs for days 7, 14, 21, and 28 determined? Calculations made using average measured concentrations of test substance?	The LC_{50} for day 21 was reported to be empirically estimated to be greater than 620 µg a.i./L, the highest mean measured concentration measured, since no concentration tested resulted in greater than or equal to 50% mortality.
Methods and data records of all chemical analyses of water quality and test substance concentrations (including method validations and reagent blanks) reported?	Yes, appendix 2 (p. 55)
Data records of holding, acclimation, test temperature, and salinity reported?	Yes

Dose Response

First Generation Survival and Reproductive Success (Offspring/Female/Reproductive Day) at Termination on Day 28:

Nominal Concentration (µg a.i./L)	Mean Measured Concentration (µg a.i./L)	Replicate	Percent Survival[a]	Percent Survival[b]	Number of Females Producing Young	Number of Offspring per Female	Reproductive Success
Control	Control	A	77	80	10	16.0	1.14
		B	77	80	10	14.3	1.02
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41	44	A	70	60	10	12.7	0.95
		B	90	95	10	13.1	0.96
		Mean	80	78			0.96
83	82	A	93	95	9	12.1	0.87
		B	73	95	9	14.4	1.05
		Mean	83	95			0.96
170	160	A	87	85	9	10.1	0.74
		B	90	95	10	10.6	0.76
		Mean	88	90			0.75[c]
330	310	A	80	75	7	12.4	0.76
		B	73	70	9	8.0	0.53
		Mean	77	73			0.64[c]
660	620	A	77	80	10	9.7	0.73
		B	63	55	10	13.0	1.06
		Mean	70	68			0.89[c]

a Study Report states that the values presented were rounded to two significant figures.

b The Study Report stated that daily survival data were presented in Appendix 3 of the report. Using that raw data, Versar calculated percent survival, but was unable to verify the values reported in the Study Report. It is unclear as to what is causing the discrepancy.

c The Study Report stated that these values were statistically different compared to control, based on Williams' Test.

Average Total Body Length at Termination on Day 28:

Nominal Concentration (µg a.i./L)	Mean Measured Concentration (µg a.i./L)	Replicate	Mean Total Body Length (mm)	
			Males	Females
Control	Control	A	6.1	6.4
		B	6.6	6.8
		Mean	6.4	6.6
41	44	A	7.3	7.0
		B	7.1	7.1
		Mean	7.2	7.1
83	82	A	7.1	6.9
		B	6.7	6.2
		Mean	6.9	6.6
170	160	A	6.5	6.8
		B	7.0	6.9
		Mean	6.8	6.9

			Mean Total Body Length (mm)	
330	310	A	7.1	6.9
		B	6.9	6.9
		Mean	7.0	6.9
660	620	A	6.8	6.9
		B	6.8	7.0
		Mean	6.8	6.9

a Study Report states that the values presented were rounded to two significant figures, however, statistical analysis was performed using unrounded values.

Dry Body Weight at Termination on Day 28:

Nominal Concentration (µg a.i./L)	Mean Measured Concentration (µg a.i./L)	Replicate	Mean Dry Body Weight (mg)	
			Males	Females
Control	Control	A	0.79	1.01
		B	0.80	1.18
		Mean	0.80	1.09
41	44	A	0.93	1.15
		B	0.93	1.19
		Mean	0.93	1.17
83	82	A	0.88	1.02
		B	0.78	1.09
		Mean	0.83	1.05
170	160	A	0.82	1.07
		B	0.87	1.17
		Mean	0.85	1.12
330	310	A	0.87	0.97
		B	0.86	1.16
		Mean	0.86	1.07
660	620	A	0.93	0.95
		B	1.01	1.05
		Mean	0.96	1.00

Statistical Results

Statistical Method:

The endpoints examined in the study included day 28 survival, growth in terms of average dry body weight and average total length) and reproduction. The MATC, LOEC and NOEC were obtained and significant differences in the percent survival were determined. Mysid data on survival, reproduction, and growth was tested for normality using Shapiro-Wilk's Test (Weber, et al., 1989), homogeneity using Barlett's Test (Horning and Weber, 1985) or Cochran's test, and were tested for statistical differences from the control using Williams' Test (Williams, 1971, 1972).

The LC_{5a} was empirically estimated to be greater then the highest concentration tested since no concentration tested results in a greater than 50% mortality and no statistical analyses were performed.

Results Synopsis: INVALID
LC_{50}: >620 µg a.i./L (day 21)
LOEC: 160 µg a.i./L
NOEC: 82 µg a.i./L
MATC: 110 µg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:
Raw data on body length, dry weight, and reproduction were not provided. The study report found mysid reproduction to be the most sensitive performance criteria. Without the raw data on offspring per female on each test date, can not verify these results for the LOEC and NOEC.

MATC
The MATC is calculated to be the geometric mean of the LOEC and NOEC. Using the report's finding (LOEC = 160 µg a.i./L and NOEC= 82 µg a.i./L), the calculated MATC as 114 mg a.i./L. Rounded to two significant figures, this value (110 mg a.i./L) agrees with that reported in the Study Report.

LC_{50} Value
The Toxanal program was used to calculate LC_{50} values. However, the program found that none of the mortality rates were greater than 50% of the control group indicating that the LC_{50} value for day 28 is greater than the highest concentration tested (620 µg a.i./L).

Results Verification Synopsis:

MATC:	114 mg a.i./L (rounded with two significant figures: 110 mg a.i./L)
LC_{50}:	>620 µg a.i./L

Could not verify the LOEC and NOEC due to the lack of raw data on individual reproduction of females per treatment day.

14. REVIEWER'S COMMENTS:

No additional comments.

DP Barcode: 321452 MRID#: 465960-13

DATA EVALUATION RECORD
AVIAN DIETARY TOXICITY TEST
GUIDELINE OPPTS 850.2200

1. **CHEMICAL:** 1 H- Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl) (93.2%) (ECONEA Technical)

   PC Code No.: 119093

2. **TEST MATERIAL:** CL322250 (Lot No. 1547-20) **Purity:** 88.2%

3. **CITATION**

Authors:	Sean P. Gallagher Kathy H. Martin Joann B. Beavers
Title:	CL322250: A Dietary LC50 Study With the Mallard duck (*Anas platyrhnchos*)
Study Completion Date:	July 8, 2005
Laboratory:	Wildlife International, Ltd. 8598 Commerce Dr. Easton, Maryland 21601
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse Belgium
Laboratory Report ID:	Janssen Study No.: AGR 1116 Wildlife International, Ltd. Project No.: 168-102
MRID No.:	465960-13

4. **REVIEWED BY:**

   Signature: Richard C. [illegible], Agronomist RAISB/BPD Date: 4/11/06

5. **APPROVED BY:**

   Signature: [illegible] Date: 4/17/06

6. **STUDY PARAMETERS**

   Scientific Name of Test Organism: *Anas platyrhynchos*
   Age of Test Organism: 10 days (Acclimation - 8 days)
   Definitive Test Duration: 11 days (Exposure - 5 days, Observation - 6 days)
   Study Method: Static
   Type of Concentrations: Nominal

7. CONCLUSIONS

Results Synopsis:

Dietary LC50: 962 ppm
95% CI: 716 to 1300 ppm
Slope: 5.468
No-mortality level: 500 ppm
NOEC: 250 ppm

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale:

C. Repairability:

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.2200:

- The average room relative humidity was 72 ± 6%, which was slightly higher than the guideline recommended upper limit of 70%.
- The photoperiod was sixteen hours of light per day during acclimation and throughout the test. The recommended photoperiod is 14 hours of light per day.
- It is not known if the avian diet was tested for contaminants periodically throughout the test.
- Observations on signs of intoxication, abnormal behavior, and mortality were not reported as being 3x on the first day of the exposure period.
- The mean measured concentrations used in the test were not provided.
- Weight of the birds that died, at the time of death, not reported.

10. SUBMISSION PURPOSE: Registration

II. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • Preferred species: either an upland game bird species, preferably the bobwhite (*Colinus virginianus*) or a wild waterfowl species, preferably the mallard (*Anas platyrhynchos*). • If bobwhite purchased, preferable that purchased as eggs which are hatched and reared in testing facility • During incubation of bobwhite quail, recommended temperature is 39°C and relative humidity is 70% • All birds used in test should be from same source and hatch	• *Anas platyrhynchos* from the same hatch were used. (pp. 10-11)
Age at beginning of test • Bobwhite quail: 10-14 days old • Mallard duck: 5-10 days old • All treatment and control birds should be same age ±1 day. • Exact age should be reported.	• All mallards were 10 days old at the initiation of the test. (p. 10)
Chicks appeared healthy and did not have excessive mortality before the test? Birds should not be used for test if more than 5% of total test population die during 72 hours preceding test	• Yes (p. 10)
Acclimation period • Acclimated to test facilities and diet for a minimum of 7 days	• The acclimation period was 8 days. (p. 13)

B. Test System

Guideline Criteria	Reported Information
Pens • Should be constructed of galvanized metal, stainless steel, or perfluorocarbon plastics • Wire mesh should be used for floors and external walls • Floor area should be at least 300 cm^2/bird for bobwhite quail and 600 cm^2/bird for mallard duck • Should be kept indoors and heated	• All birds were housed indoors in thermostatically controlled brooding pens. Each pen had floor space that measured approximately 62 x 92 cm (5704 cm2). Ceiling height was approximately 25.5 cm. External walls, ceilings, and floors were constructed of vinyl coated wire grid. (p. 13)
Room temperature • 22-38°C	• During the test, the average temperature in the brooding compartment was 30.4 ± 1.2°C. The average room temperature was 23.9 ± 0.6°C (p. 14).
Relative humidity • 45-70%	• The average room relative humidity was 72 ± 6% (p. 14).
Photoperiod • Recommended 14 hours light/10 hours dark • Continuous lighting is acceptable	• The photoperiod was sixteen hours of light per day during acclimation and throughout the test (p. 14).
Diet • A commercial diet for game birds or duck starter mash should be used • Only clean, unmedicated water should be offered during 96 hours preceding test period • Diets should be analyzed periodically for contaminants • Nutrient analysis and list of ingredients in diet should be included in report • Clean water should be available *ad libitum*; if water pans or bowls used water should be changed at least once a day	• All test birds were fed a game bird ration *ad libitum* formulated to Wildlife International, Ltd's specifications. (p. 11) • Water from the town of Easton public water supply was provided *ad libitum*. (p. 11) • The birds received no form of antibiotic medication during acclimation to the test. (p. 11) • The diet formulation was provided in Appendix II. The analysis of the formulation did not list any sources of contamination, however, periodic testing of contaminates was not indicated. (p. 24) • Samples of the test diets were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance diet. (p. 11)

C. Test Design

Guideline Criteria	Reported Information
Range finding test • Should be conducted • Generally, groups of a few birds fed 3 to 5 widely spaced concentrations for 5 days • Concentration series of 5, 50, 500, and 5,000 ppm suggested	• The dietary concentrations were established based upon known toxicity data and results of a range-finding test that demonstrated 60% mortality at a dietary concentration of 1000 ppm a.i. (p. 9)
Test Concentrations • Minimum of 5 concentrations spaced geometrically • Recommended spacing is for each concentration to be at least 60% of next highest dose • At least one concentration should kill more than 50% and at least one concentration should kill less than 50% • Treated diets should be analyzed to confirm proper dietary concentration of test substance–should be conducted at beginning of exposure period with samples from high, middle and low concentrations	• Nominal dietary test concentrations used in this study were 0, 62.5, 125, 250, 500, 1000 and 2000 ppm ai CL322250. (p. 11) • Test concentrations of 0, 62.5, 125, 250 and 500 ppm resulted in zero deaths. Test concentrations of 1000 ppm and 2000 ppm resulted in a maximum of 7 (70%) and 9 (90%) deaths, respectively, by day 5. (pp. 19, 20) • Verification samples were collected from each concentration level at day zero and day 5 to assess the stability of the test substance. (p. 12)
Controls • Concurrent control group required • Should be from same hatch as those used in treatments • Kept under same environmental conditions	• A control group of 30 mallard ducklings (5 ducklings per pen) were studied concurrently and kept under the same environmental conditions. (pp. 9, 11, 14)
Number of birds per group • Minimum of 10 per test concentration • Minimum of 20 for negative or carrier controls; 30 or more control birds is preferred	• Ten mallard ducklings were assigned to each of the treatment groups by indiscriminate draw. (p. 9) • A control group of 30 mallard ducklings (5 ducklings per pen) were studied concurrently. (p. 9)

Guideline Criteria	Reported Information
Test Substance • Should be mixed in diet evenly • Should be added without use of diluent; if needed preferred diluent is distilled water or if substance is not water soluble, reagent grade evaporative diluent (e.g., acetone or methylene chloride) • Other possible diluents: corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic • If diluent used, should not comprise more than 2% by weight of treated diet • Diets can be mixed by commercial, mechanical food mixers and may be mixed under a hood • Should be mixed freshly just prior to beginning of test	• Test diets were prepared by mixing the test substance with the feed on a Hobart (Model Number AS200T) mixer. (p. 11) • It was not reported if a diluent was used in the diet preparation. • Homogeneity of the test substance in the diet was evaluated from six samples at the 62.5 ppm and 2000 ppm test diet preparations at day 0. (pp. 11, 12)
Test Acceptability • No more than 10% of control birds die • Evidence provided that test concentrations were at least 80% of nominal for first 5 days of test period • Lowest treatment level did not result in compound-related mortality or other observable effects	• Zero control birds died during the test. (pp. 19, 20) • The test concentrations at day 5 ranged from 95-100% of the mean day zero concentrations. (p. 32) • Zero birds at the 62.5 ppm concentration died during the test. (pp. 19, 20)
Test durations • 5 days with treated feed and at least 3 days observation with "clean" feed • If any test birds die during 2nd or 3rd day of postexposure period, test period should be extended until 2 successive mortality-free days and 1 day free of toxic signs occur or until 21 days after beginning of test (whichever comes first)	• During the test, each group was fed the appropriate treated diet for five days followed by six days of receiving untreated basal diet. (pp. 9, 10) • All mortalities occurred during the first five days (exposure period). (p. 19)

Guideline Criteria	Reported Information
Observations • Signs of intoxication, abnormal behavior and mortality should be recorded and reported by dose level and by day • Should be made at a minimum 3x on the first day of exposure • Should be made at least twice during remainder of test period; twice daily observations recommended • Average body weights should be reported at beginning and end of normal 3-day postexposure period • Average food consumption should be measured either daily or every other day in controls and pens with second lowest and second highest concentration levels; for other pens should be measured for both the exposure period and the normal 3-day postexposure period	• During the test, all birds were observed at least twice daily. A record was maintained of all signs of toxicity and abnormal behaviors. It was not reported whether observations were made at least 3 times on the first day of exposure. (p. 14) • Individual body weights were measured at the initiation of the test (day 0), on day 5, day 8 and at the termination of the test on day 11. (p. 14) • Average feed consumption values were determined daily during the exposure period (days 0-5) and twice during the post-exposure observation period (days 6-8 and 9-11) by pen for each treatment group and the control. (p. 14)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	• Yes (pp. 3, 4)
Name of test, sponsor, test laboratory and location, principal investigators and actual dates of beginning and end of test reported?	• Yes (cover, p. 8)
Name of test species, age, average body weights and individual body weights of all birds that die during test reported?	• Yes, except individual weights of all birds that died were not reported. (pp. 10, 14)
Description of housing conditions (type, size and material of pen, temperatures, humidity, photoperiod and lighting intensity) reported?	• Yes. (pp. 13, 14)
Detailed description of diet (source, diluents, supplements, if used) reported? Nutrient analysis of diet included?	• Yes. (pp. 11, 24)
Detailed description of test substance including chemical name, source, composition, physical/chemical properties reported?	• Yes. (p. 23)

Guideline Criteria	Reported Information
Number of concentrations used, nominal and measured concentrations, number of birds per concentration and for controls reported?	• The number of concentrations used and birds per concentration were reported; however, the mean measured concentrations were not provided. (p. 11)
Acclimation procedures reported?	• Yes. (p. 9)
Frequency, duration and methods of observation reported?	• Yes. (pp. 14, 40-63)
Signs of toxicity (if any) were described?	• Yes. (pp. 15, 16)
Raw data included?	• Yes. (pp. 19-22, 40-73)

Dose Response

There were no mortalities in the control group or in test concentration groups 62.5, 125, 250, or 500 ppm. There was 70% mortality in the 1000 ppm treatment group and 90% mortality in the 2000 ppm treatment group.

Mortality

Nominal Concen. (ppm)	No. of Birds	Cumulative Mortality Day of Study											
		0	1	2	3	4	5	6	7	8	9	10	11
0	30	0	0	0	0	0	0	0	0	0	0	0	0
62.5	10	0	0	0	0	0	0	0	0	0	0	0	0
125	10	0	0	0	0	0	0	0	0	0	0	0	0
250	10	0	0	0	0	0	0	0	0	0	0	0	0
500	10	0	0	0	0	0	0	0	0	0	0	0	0
1000	10	0	0	2	4	6	7	7	7	7	7	7	7
2000	10	0	0	5	6	8	9	9	9	9	9	9	9

Statistical Results

Statistical Method: The statistical methods used were not provided.

Results Synopsis: Dietary LC50: 962 ppm (95% CI of 716 to 1300 ppm)
Slope: 5.468
Chi-Square: 2.599
No-mortality level: 500 ppm
NOEC: 250 ppm

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: The dietaryLC50 was calculated using the Toxanal program which provides results from three different statistical tests: the binomial method, the moving

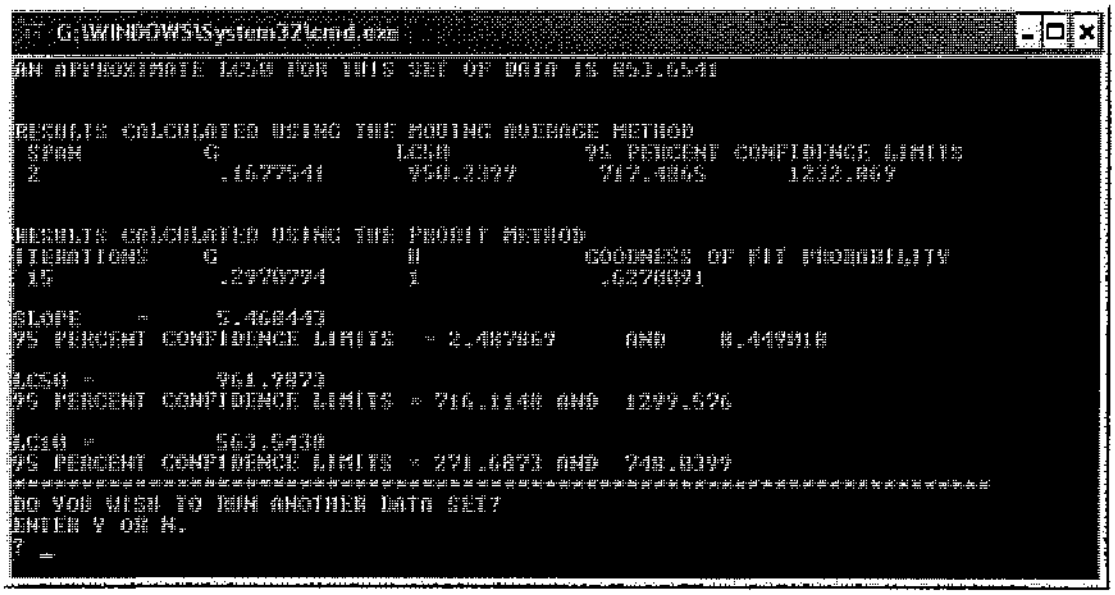
average method, and the probit method. The NOEC was determined empirically from a review of both the mortality data and the symptoms data.

Results Verification Synopsis:

Dietary LC50: 962 ppm
95% CI: 716 to 1300 ppm
Slope: 5.468
No-mortality level: 500 ppm
NOEC: 250 ppm

14. REVIEWER'S COMMENTS: No additional comments.

Determination of the LD_{50}:



DP Barcode: 321453 MRID No: 465960-14

DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** ~~ECONEA Technical~~ CL 322, 250 Hawk PC Code No.: 119093

2. **TEST MATERIAL:** CL322,250 Purity: 92.6%

3. **CITATION**

Author:	Hoberg, James R.
Title:	CL322,250—Acute Toxicity to the Marine Diatom, *Skeletonema costatum*, Under Static Conditions
Study Completion Date:	March 17, 2005
Laboratory:	Springborn Smithers Laboratories, 790 Main St. Wareham MA 02571-1075
Sponsor:	Janssen Pharmaceutica NV, Plant and Material Protection Division, Turnhoutseweg 30, B-2340 Beerse, Belgium
Laboratory Report ID:	13751.6147
DP Barcode:	3214543
MRID No.:	465960-14

4. **REVIEWED BY:**

Signature: Date: **1/19/06**
David C. Bays, RASSB, AD (7510C)

5. **APPROVED BY:**

Signature:
Rick Petrie, Team 3 Leader, RASSB, AD (7510C) Date: **1/19/06**
Kathryn Montague, Acting Team 1 Leader, RASSB, AD (7510C)

6. **STUDY PARAMETERS**

Definitive Test Duration: 96-hour
Type of Concentrations: Nominal

7. **CONCLUSIONS**

Results Synopsis: A significant reduction in cell density was detected in treatment levels 0.13 mg a.i./L. Because the Williams' test did not determine a NOEC, Bonferroni's Test was used. Bonferroni's Test determined a significant reduction in cell density in the 0.13 and 1.0 mg a.i./L treatment levels. However,the next two higher

treatment levels (0.23 and 0.50 mg a.i./L) were not affected and the reduction in cell density was not considered treatment-related. Based on Bonferroni's Test the NOEC was determined to be 0.50 mg a.i./L. The 96-hr EC50 value was determined to be 0.66 mg a.i./L, with 95% confidence intervals of 0.60 to 0.70 mg a.i./L.

Verified Results Synopsis: The results of the verification calculations using Dunnet's test and Bonferroni's test showed statistically significant differences in the 1.0 mg/L dose group only. This differs from the results obtained by the study author using Williams' test (statistically significant differences at all analyzed treatment levels) and Bonferroni's test (statistically significant differences in the 0.13 and 1.0 mg/L dose groups). It is unclear, without more information regarding the study author's calculations, why this discrepancy exists. No other calculation errors were found in the review of statistical calculations.

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: Provide the missing information (see section 9 below)

C. Repairability: If the registrant provides the missing information, then the study can be upgraded to core.

9. GUIDELINE DEVIATIONS

The following guideline deviations were based on EPA OPPTS Guideline 850.5400:

- The light intensity fell outside the range of 4.3 k Lx ± 10% on days 2 and 3, when the light intensity at three vessels was measured to be 450 to 460 footcandles (4.9 to 5.0 K lx).
- The following items were not reported in the study report:
  - Sterilization/cleaning practices
  - Water solubility
  - Physical/chemical properties of the chemical, including saturation concentration
  - ~~The maximum labeled rate~~ NCork
- ~~Only two replicates per dose/control group were used in the range-finding test, instead of three.~~ NCork
- Doses selected for the main test progressed by factors of 2.5-2.6 times, rather than 1.5-2 times.
- ~~No positive control was used.~~ NCork
- Although five treatment levels were created, the 0.063 mg/L data was excluded from statistical analysis because there were indications that the test solution was not fortified at the desired concentration.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species •*Selenastrum capricornatum (Raphidacelis subcapitata)* •*Skeletanema castatum* •*Anabaena flas-aqnae* •*Navicula pelliculasa*	*Skeletanema castatum* was used.
Initial Number of Cells •10,000 cells/mL (*Selenastrum, Anabaena, Navicula)* •77,000 cells/mL (*Skeletanema*)	Approximately 77,000 cells/mL. p15
Stock Culture •3 to 7 days old	Three days. p13
Nutrients •Standard formula (ASTM E1218-20) •pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletanema*) •Freshly prepared	• Sterile medium used • pH=8.1± 0.1

B. Test System

Guideline Criteria	Reported Information
Solvent Upper limit - 0.5 mL/L	• 0.1 mL/. Lp15
Temperature •24° ± 2°C (*Selenastrum, Navicula, Anabaena*) •20° ± 2°C (*Skeletanema*) •Recorded hourly	• 20°C±2°C. p23,27 • Temperature recarded continuously. p16
Light Intensity •4.3 K lx (±10%) (*Selenastrum, Skeletanema, Navicula*) •2.2 K lx (±10%) (*Anabaena*) •Photosynthetically active radiation approx. 66.5 ± 10% μEin/m^2/sec	• 3.9 to 4.7 K lx, except at the 24-hr observation period, when the light intensity at three vessels was measured to be 450 to 460 footcandles (4.9 to 5.0 K lx). p23,27
Photoperiod •14-hr light/10-hr dark (*Skeletonema*) •Continuous (*Selenastrum, Navicula, Anabaena*)	14-hr light/10-hr dark used. p16

Guideline Criteria	Reported Information
pH •pH of nutrient medium: 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*) 8.1 ± 0.1 (*Skeletonema*) •Measured at beginning and end of test	• Nutrient medium pH = 8.1±0.1. p13 • Measured at beginning and end of test. p27
Oscillation Rates •100 cycles/min (*Selenastrum*) •60 cycles/min (*Skeletonema*)	• 60±10 rpm. p13
Test Containers •125-500 mL Erlenmeyer flasks •Cleaned/sterilized (solvent and acid) and conditioned •Test solution volume ≤ 50% of flask volume	• 250 mL Erlenmeyer flasks. p15 • Conditioned, but sterilization/cleaning not reported • Test solution volume = 100 mL. p15
Dilution Water •Sufficient quality (e.g., ASTM Type I) •Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	• Artificially enriched seawater used (salinity = 30±2 g/L). p13

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test •Water solubility and physical-chemical properties of test chemical determined? •Validated analytical method developed? •Expose algae to widely spaced (e.g. log interval) chemical concentration series •Lowest value should be at detection limit •Upper value, for water soluble compounds, should be at saturation concentration •Minimum of 3 replicates •Algae should be exposed for 96 hours •If highest concentration (saturation concentration or 100 mg/L) results in <50% reduction in growth, definitive test may not be necessary •If lowest concentration (detection limit) results in >50% reduction, definitive test necessary	• Water solubility, physical/chemical properties could not be found in the study report. p19 • Validated method. p48 • Log intervals used. p19 • Lowest concentration of range-finding test (0.0010 mg a.i./L).p19; below detection limit (0.0125 mg a.i./L).p54 Saturation concentration not reported. • Two replicates per dose/control group. p19 • 96 hours of exposure • Definitive test justified based on results from range finding test
Dose Range •1.5X -2X progression	• 2.0X progression calculated from doses

Doses •5 or more concentrations of test substance in a geometric series •> 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	• 5 doses in a geometric series; however, one dose group was excluded from statistical analysis because there were indications that the test solution was not fortified at the desired concentration .p22 • 100% inhibition at highest doses. p27
Controls •Negative and/or solvent each test •Positive - zinc chloride (periodically)	• Negative and solvent controls used • No positive control
Replicates Per Dose •3 or more (4 or more for *Navicula*)	• Three replicates/dose. p15
Duration of Test •96-hr	• 96 hour duration.
Growth •Logarithmic growth (controls) by 96-hr or repeat test (increase by a factor of 16) •1.5 x 10^6 cells/mL (*Skeletonema*) •3.5 x 10^6 cells/mL (*Selenastrum*)	• Increase by more than a factor of 16. 1.49x10^6 cell/mL at 96 hrs. p30
•Daily Observations?	Yes. p16
Method of Observations •Direct - microscopic cell count of at least 400 cells/flask •Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count •Qualitative and descriptive	Direct method used. p15 At least 400 cells counted. p16
Cell Separation •Syringe ultrasonic bath, or blender; limited sonification (*Anabaena*) •Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula*)	No report of filament-breaking could be found in the study report.
•Algistatic and algicidal effects differentiated?	Yes. p16
•Maximum Labeled Rate	It is unclear if the maximum labeled rate was used.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	Yes. p13

Growth in controls reported?	Yes. p30
Description of test system and test design incinded?	Yes
Initiai and final chemical concentrations and pH measured?	Yes
Initial, 24-, 48-, 72- and 96-hr cell densities measnred? % of inhibition or growth and other adverse effects reported?	Yes
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	Yes
Raw data incinded?	Yes. p30
Methods and data records reported?	Yes. p18, appendix 2
Statistical Analysis •Mean and standard deviation calculated and plotted? •Goodness-of-fit determined?	Yes.

Dose Response

Nominal Concentration (mg/L) -	Initial Measured Concentration (mg/L)	Final Measured Concentration (mg/L)	Cell Density at 96 hrs (x 10^4 cells/mL) -	% Inhibition (reduction in growth rate compared with Pooled Control / Solvent Control)	pH	
					0-hr	96-hr
Control	<0.014	<0.015	145.58±26.07	NA	8.0	9.0
Solvent Control	<0.014	<0.015	149.67±19.91	NA	8.0	8.9
Pooled Control	NA	NA	147.63±20.86	NA	NA	NA
0.063	<0.014	<0.015	124.75±27.67	15	8.0	8.9
0.13	0.13	0.13	99.25±36.93	33	8.0	9.0
0.25	0.22	0.25	113.75±24.54	23	8.0	8.9
0.50	0.51	0.48	115.50±11.91	22	8.0	8.9
1.0	1.1	1.0	0.50±0.50	100	8.0	8.0

a The 0.063 mg/L dose group was excluded from statistical analysis because there were indications that the test solution was not fortified at the desired concentration

Statistical Resuits

Statistical Method: A t-test was used to compare the daily cell density of the control to the solvent control. The solvent control was used for comparison to treatment data if a significant difference was determined; otherwise, the control and solvent control data were pooled and used for comparison. EC50 values were calculated using TOXSTAT. The NOEC was determined by determining the highest test concentration which demonstrated no statistically adverse effect (p 0.05). Normality was checked using Shapiro-Wilks' Test, and homogeneity of variance was checked using Bartlett's Test. If the data sets passed the test for homogeneity and normality, then Williams' Test was used to determine the NOEC. p18

Results Synopsis: Because no significant difference was determined between the control and solvent control data, the pooled control and solvent control data were used for comparison to treatment data. The cell density data were found to be normally distributed and have homogeneity of variance; therefore, the Williams' Test was used to determine treatment-related effects. A significant reduction in cell density was detected in treatment levels 0.13 mg a.i./L. Because the Williams' test did not determine a NOEC, Bonferroni's Test was used. Bonferroni's Test determined a significant reduction in cell density in the 0.13 and 1.0 mg a.i./L treatment levels. However,the next two higher treatment levels (0.23 and 0.50 mg a.i./L) were not affected and the reduction in cell density was not considered treatment-related. Based on Bonferroni's Test the NOEC was determined to be 0.50 mg a.i./L. The 96-hr EC50 value was determined to be 0.66 mg a.i./L, with 95% confidence intervals of 0.60 to 0.70 mg a.i./L.

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Calculations of cell density averages and standard deviations were checked by Versar for accuracy. EC50 calculations were inspected for reasonableness with respect to the raw data. In order to verify calculations of the 96-hr NOEC, the Dunnet's test and Bonferroni's test ($p<0.05$) was performed on the cell density data. Data from the 0.063 mg a.i/L dose group were excluded from analysis, to be consistent with the study report. **Results Verification Synopsis:** The results of the verification calculations using Dunnet's test and Bonferroni's test showed statistically significant differences in the 1.0 mg/L dose group only. This differs from the results obtained by the study author using Williams' test (statistically significant differences at all analyzed treatment levels) and Bonferroni's test (statistically significant differences in the 0.13 and 1.0 mg/L dose groups). It is unclear, without more information regarding the study author's calculations, why this discrepancy exists. No other calculation errors were found in the review of statistical calculations.

14. REVIEWER'S COMMENTS:

The following guideline deviations were found in the study report:

- The light intensity fell outside the range of 4.3 k Lx ± 10% on days 2 and 3, when the light intensity at three vessels was measured to be 450 to 460 footcandles (4.9 to 5.0 K lx).
- The following items were not reported in the study report:
  - Sterilization/cleaning practices
  - Water solubility
  - Physical/chemical properties of the chemical, including saturation concentration
  - ~~The maximum labeled rate~~ MCook
- ~~Only two replicates per dose/control group were used in the range-finding test, instead of three.~~ MCook
- Doses selected for the main test progressed by factors of 2.5-2.6 times, rather than 1.5-2 times.
- ~~No positive control was used.~~ MCook
- Although five treatment levels were created, the 0.063 mg/L data was excluded from statistical analysis

because there were indications that the test solution was not fortified at the desired concentration.

ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

August 17, 2006

MEMORANDUM

SUBJECT: Environmental Fate Assessment of Econea™ Technical for New Chemical Registration

Case No.: **DP Barcode:** 330789

FROM: Srinivas Gowda, Microbiologist/Chemist Srinivas Gowda
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

James Breithaupt, Agronomist James Breithaupt
Environmental Risk Branch II
Environmental Fate and Effects Division (7507P)

TO: Marshall Swindell, Team Leader
Karen Leavy, Risk Manager Reviewer
Regulatory Management Branch I
Antimicrobials Division (7510P)

THRU: Siroos Mostaghimi, Team Leader, Team one Siroos Mostaghimi
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Norman Cook, Branch Chief
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Chemical Name	PC Code	CAS#	Common Name
1H-Pyrrole-3-Carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-	119093	122454-29-9	Econea™

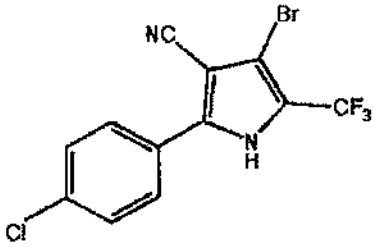
Environmental Fate Science Chapter and Fate Assessment on Econea™ Technical is submitted for New Chemical Registration.

ECONEA™ Technical
ENVIRONMENTAL FATE SCIENCE CHAPTER

EXECUTIVE SUMMARY

ECONEA™ Technical is an anti-fouling preservative that contains 93.2% of the active ingredient 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as AC303268 (common name), R107894, or AF028. It is used for formulation into anti-fouling products for control of hard fouling organisms such as barnacles, mussels, and polychaetes found on the hulls of boats and vessels, as well as on marine structures.

AC303268 is an off-white powder that is practically insoluble in water. The chemical structure of AC303268 is as follows:



A 45-day aqueous availability study shows that AC303268 may be released from paint into surface waters. The average leach rate of AC303268 in seawater (from Sigma Nexxium 20 Paint), between day 28 and day 45, was 8.00 $\mu g/cm^2$/day, with an average cumulative release of 12.9 $\mu g/cm^2$ through day 1 and 454 $\mu g/cm^2$ through day 45. Any AC303268 released into water is rapidly hydrolyzed, primarily at higher temperatures and pH values to one major degradate, CL 322,250 (parent minus fluorines and remaining carbon hydrated). Hydrolytically, at pH 5 and 10°C, the half-life of AC303268 is 168 days, as opposed to 15 days at pH 5 and 25°C, and less than 3 days at pH 7 and pH 9 (10 and 25°C). In seawater, AC303268 hydrolyzes with a half-life of less than 1 day at 10 and 25°C. The degradate CL 322,250 does not degrade at any pH or temperature due to hydrolysis. Based on its rapid hydrolysis, AC303268 may not pose a concern as a contaminant in surface waters. However, because of its stability, CL 322,250 may be a concern.

Aerobic and anaerobic aquatic metabolism continue to degrade AC303268, decreasing the threat of surface water contamination. In an aerobic aquatic metabolism study, AC303268 degraded with estimated half-lives of 3-7 days and less than 1 day in freshwater and marine test systems, respectively. Two major degradates, CL 322,250 and debrominated CL 322,250 (found only in marine water), were identified and the majority of the residues were found in the aqueous layer, as opposed to the sediment. CL 322,250 was stable in the freshwater test system and degraded with a half-life of 288 days in the marine test system. Under anaerobic conditions, AC303268 degraded into the same two degradates in both the freshwater and marine test systems, and were again found primarily in the aqueous layer. Half-lives were similar at 10 days in the freshwater test system and 0.03 days in the marine test system. However, the percent of degradate present during different periods of time varies with the type of metabolism. In

addition, CL 322,250 continued to degrade (half-lives 31 and 22 days) to debrominated 322,250 in the freshwater and marine test system under anaerobic conditions.

AC303268 is also expected to absorb to suspended solids and sediments in surface waters, thereby reducing its concentration in surface waters. In a batch equilibrium study, an average of 98.89 and 98.38% of the applied amount was absorbed in the freshwater soils (sandy loam and silt loam), respectively. In marine soils (sand and loam), an average of 83.18% and 97.48% was absorbed, respectively. Average adsorption K_d values ranged from 450 to 335 ml/g in the freshwater soils and from 26 to 196 ml/g in the marine soils. Corresponding K_{oc} values were 20440 to 16733 and 3582 to 5588 ml/g. Desorption K_d and K_{oc} values were higher than those obtained for adsorption. Adorption coefficients for the degradate CL 322,250 indicate that it is also absorbed to suspended solids and sediments.

The estimated Log Kow for parent Econea™ (AC 303268) is 3.0, and the estimated Log Kow values for the primary degradate (CL 322,250) are 1.66 in freshwater and 0.55 in salt water. Parent Econea™ generally degrades quickly in water to CL 322,250, and therefore bioconcentration was modeled using the primary degradate. A Log Kow of less than 3.0 (Kow <1000) would be indicative of bioconcentration that is below our level of concern. Therefore, significant bioconcentration of CL 322,250 in freshwater and saltwater fish is not likely to occur. The Agency has estimated bioconcentration factors (BCFs) of 11X (pH 6) and 3X (pH8) in freshwater and seawater, respectively.

I. Environmental Fate Assessment

A. Abiotic

In a hydrolysis study conducted under abiotic and buffered conditions, AC303268 (R107894) was rapidly hydrolyzed, primarily at higher temperatures and pH values. The study was conducted in the dark at temperatures of 10 and 25 ± 1°C for up to 30 days at pH 5, pH 7, pH 9, and in synthetic seawater (pH 8-nonbuffered). At 25°C, AC303268 hydrolyzed with respective half-lives of 15 days, 8 hours, 2 hours and 3 hours at pH 5, pH 7, pH 9 and in seawater. Half-lives were 168 days, 69 hours, 12 hours and 15 hours at 10°C. Hydrolysis produced CL 322,250 as the major degradate, which was present in all solutions analyzed with the exception of the pH 5 solution at 10°C. Traces of CL 325,195 (hydrated and debrominated parent) were also identified. Only minor hydrolytic products were formed in the pH 5 solution at 10°C. At 10°C, CL 322,250 was present at a maximum concentration of 72.7% of the applied (day 21) and at a maximum concentration of 96.2% (day 30) in the pH 7 and pH 9 buffered solutions, respectively. In seawater, a maximum concentration of 95.8% of the applied was observed on day 21. At 25°C, CL 322,250 was present at maximum concentrations of 73.9% (day 30), 72.4% (day 7), 96.9% (day 7), and 96.3% (24 hrs) of the applied in the pH 5, pH 7, pH 9 and seawater test solutions, respectively. The hydrolysis guideline requirements (OPPTS 161-1) for ECONEA™ Technical have been fulfilled by this study (MRID Nos. 456739-08 and 456739-09).

The Agency also performed regression analyses using the data presented in the study to

estimate the half-lives of the parent compound (AC303268) and the major degradate (CL 322,250). In freshwater, half-lives of the parent compound ranged from 177 days at pH 5 and 10°C, 15 days at pH 5 and 25°C, to 3 days in the pH 7 and 9 buffered solutions at 10 and 25°C. The half-lives were less than 1 day in the seawater (pH 8) at 10 and 25°C. While degradation of the parent compound occurred, CL 322,250 did not degrade at any pH or temperature.

A 45-day aqueous availability study determined the rate at which two active ingredients, one of which was AC303268 (AF028), are released from Sigma Nexxium 20 Paint. The paint was applied to polycarbonate cylinders which were immersed in a tank with continuously pumped synthetic seawater. The average leach rate between day 28 and day 45 was 8.00 $\mu g/cm^2$/day. The average cumulative release was 12.9 $\mu g/cm^2$ through day 1 and 454 $\mu g/cm^2$ through day 45. The study reflects the guideline specified for the ASTM Standard Test Method D5108-90 for aqueous availability (MRID No. 456732-01).

B. Biotic

The aerobic metabolism of AC303268 (R107894) was studied in a natural freshwater/sediment system (water pH 6.5, silt loam, organic carbon 2.5%) and a natural marine water/sediment system (water pH 8.04, sandy loam, organic carbon 0.8%). The study was conducted for 30 days in the dark at 21°C. AC303268 was applied at the rate of 0.5 mg/L. The estimated half-life (based on visual inspection of the data) in the freshwater system was between 3 and 7 days. In the marine system, the half-life was estimated as being less than 1 day. The two major degradates identified were CL 322,250 and debrominated CL 322,250. There were also four minor degradates. A higher percentage of both the parent compound and the degradates was found in the aqueous phase as opposed to the sediment. The major degradate identified in the freshwater was CL 322,250, with a maximum concentration of 48.2% of the applied on day 7. The major degradate in the freshwater sediment was also CL 322,250, with a maximum concentration of 7.85% of the applied observed on the last day (30^{th}) of the study. There were two major degradates identified in the marine water and sediment. CL 322,250 and debrominated CL 322,250 were detected in the marine water at maximum concentrations of 71.9% and 19.5% of the applied, respectively, on days 7 and 30 of the study. In the marine sediment, CL 322,250 and debrominated CL 322,250 were detected at maximum concentrations of 5.22% and 10.8% of the applied on days 15 and 30, respectively. The aerobic aquatic metabolism guideline requirements (OPPTS 162-4) for ECONEA™ Technical have been fulfilled by this study (MRID Nos. 456739-11 and 456739-12.

The Agency also performed regression analyses using the data presented in the study to estimate the half-lives of the parent compound (AC303268) and the major degradate (CL 322,250). In the freshwater system, the half-life of the parent compound was estimated at 12 days. CL 322,250 was stable. The half-lives were 0.62 and 288 days, respectively, for the parent compound and CL 322,250 in the marine system, where CL 322,250 farther degraded to debrominated CL 322,250.

A study of the anaerobic metabolism of AC303268 (R107894) was also performed. The

study was conducted in a natural freshwater/sediment system (water pH 5.8, silt loam, organic carbon 2.5%) and a marine water/sediment system (water pH 7.7, loamy sand), organic carbon 0.8%) for 52 weeks in the dark at 21°C. AC303268 was applied at the rate of 69 µg/L. Based on modeling, AC303268 degraded with a half-life of 10 days in the freshwater system and a half-life of 0.03 days in the marine system. The major degradates of both the freshwater system and the marine system were CL 322,250 and CL 325,195 (hydrated and debrominated parent). Seven unknown minor degradates were also detected. In the water of freshwater test system, CL 322,250 was present at a maximum concentration of 44.10% of the applied on day 14. CL 325,195 was below the detection limit throughout the study period. In the water of the marine test system, CL 322,250 and CL 325,195 were at maximum concentrations of 60.34% and 6.64% of the applied, respectively, on day 3. Maximum concentrations in the sediment of the freshwater system were 10.05% of the applied for CL 322,250 and 1.29% of the applied for CL 325,195, observed on day 14 and day 7, respectively. In the marine system, maximum concentrations in the sediment were 16.35% of the applied on day 7 and 1.39% of the applied at time 0. This study satisfies the anaerobic metabolism guideline requirements for ECONEA™ Technical (OPPTS 162-3) (MRID No. 456739-10).

The Agency also performed regression analyses using the data presented in the study to estimate the half-lives of the parent compound (AC303268) and the major degradate (CL 322,250). In the freshwater system, half-lives of the parent and the major degradate were 29 and 31 days, respectively. The half-lives were 0.68 and 22 days, respectively, for the parent compound and CL 322,250 in the marine system.

The adsorption/desorption characteristics of AC303268 (R107894) were studied in two freshwater soils, sandy loam and silt loam, and two marine soils, sand and loam. Results of the study indicate that AC303268 is strongly absorbed to soil. After 4 hrs of equilibration for sandy loam, silt loam, loam and 8 hrs of equilibration for sand, an average of 98.89, 98.38, 97.48 and 83.18% of the applied amount was adsorbed, respectively. Average adsorption K_d values were 450, 335, 26, and 196 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. The average adsorption K_{oc} values were 20440, 16733, 3582, and 5588 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. K_f values were 446, 349, 22, and 183 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. At the end of the desorption phase, 0.84, 0.88, 9.62, and 1.63% of the adsorbed AC303268 was desorbed in the sandy loam, silt loam, sand, and loam soils, respectively. Average desorption K_d values were 599, 568, 40, and 299 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. The average desorption K_{oc} values were 27229, 28353, 5658, and 8543 ml/g for sandy loam, silt loam, sand, and loam soils, respectively. Desorption K_d and K_{oc} values were higher than those obtained for adsorption. The adsorption/desorption guidelines requirements (OPPTS 163-1) for ECONEA™ Technical have been fulfilled by this study (MRID No. 456739-13).

The adsorption/desorption properties of the parent compound (AC 303268) and the major degradate (CL 322,250) were also estimated by the Agency using the data presented in the study. Adsorption K_f values (parent compound) of 446 and 349 ml/g were estimated for the freshwater soils (sandy loam and silt loam) and K_f values of 22 and 183 ml/g were estimated for the marine soils (sand and loam). Corresponding K_{oc} values were 20273, 17450, 3143 and 5229 ml/g. No

correlation with clay, organic matter, or pH was noted. The adsorption and desorption coefficients of the degradate CL 322,250 were similar. Adsorption K_f values of 189 and 357 were estimated for the freshwater soils. The adsorption K_f values in marine soils were 14 and 119. Corresponding K_{oc} values were 8591, 17850, 2000, and 3400 ml/g. As with the parent compound, desorption K_f and K_{oc} values for CL 322,250 were higher in all soils.

The bioconcentration of the major degradate CL 322,250 in freshwater and seawater was estimated by Agency based on the log octanol/water partition coefficient (Log Kow). Using equations presented in the OECD TG 305 Guideline, bioconcentration factors of 11X (pH 6) and 3X (pH8) were predicted in freshwater and saltwater fish, respectively.

APPENDIX

Environmental Fate Data for ECONEA™ Technical

A. Environmental Fate Guideline Studies

1. Hydrolysis (Guideline Number OPPTS 161-1, MRID No. 456739-08 and 456739-09)

This hydrolysis study, submitted under MRID Nos. 456739-08 and 456739-09, was reviewed by the Agency and found to be acceptable for the active ingredient, 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as R107894. The hydrolysis data requirements for ECONEA™ Technical have been fulfilled.

In the main part of the study (MRID No. 456739-08), hydrolysis of radiolabelled [^{14}C]-R107894, at a nominal concentration of 0.5 µg/g, was studied. The test solutions were incubated in the dark at nominal temperatures of 10 and 25 ± 1EC for up to 30 days in 0.01 M citrate buffer (pH 5), 0.01 M TRIS-maleic acid buffer (pH 7), 0.01 M borate buffer (pH 9) and seawater. Samples were analyzed at 0, 3, 5, 12, and 24 hours and at 2, 3, 4, 7, 10, 14, 21, and 30 days. Radioactivity was quantified by direct injection using a liquid scintillation analyzer (Packard Tri-carb 1600 TR) and identification of the transformation products was conducted using high performance liquid chromatography (HPLC) (Hewlett-Packard 1050 series HPLC and a Berthold LB 507A radioactivity monitor) and thin layer chromatography (TLC) (Molecular Dynamics phosphor imager).

The radioactive balance was 87.2 ± 11.8%, 88.6 ± 2.0%, 102.2 ± 1.0%, and 87.1 ± 0.8% of the applied at pH 5, pH 7, pH 9, and seawater at 10EC, respectively. At test termination, the concentration of the parent compound at 10EC decreased from 94.0% at day 0 to 80.9% of the initial at pH 5, decreased from 77.9% of the initial at day 0 to not detectable by day 21 at pH 7, decreased from 51.4% of the initial at day 0 to not detectable by day 4 at pH 9, and decreased from 54.9% of the initial at day 0 to not detectable by day 4 in seawater. At pH 5 (10EC) there were no major transformation products detected. At pH 7 (10EC), the major transformation products detected were CL 322,250 and Unknown B with maximum concentrations of 72.7% and 25.8% of the applied observed on the 21st and 30th days of incubation, respectively. At pH 9, the major transformation product detected was CL 322,250, with a maximum concentration of 96.2% of the applied amount observed on the 30th day of incubation. In seawater, the major transformation product detected was CL 322,250 with a maximum concentration of 95.8% of the applied amount observed on the 21st day of incubation. The minor transformation products detected at pH 5 were CL 322,250; CL 325,195; Unknown C; Unknown D; and Unknown G formed at maximum concentrations of 9.4, 4.2, 3.1, 2.6, and 0.61% of the applied, respectively. The minor transformation products detected at pH 7 were CL 325,195; Unknown A; Unknown C; and Unknown D formed at maximum concentrations of 1.6, 5.8, 1.8, and 1.9% of the applied, respectively. The minor transformation products detected at pH 9 were CL 325,195; Unknown A; Unknown B; Unknown C; and Unknown D formed at maximum concentrations of

2.7, 1.4, 2.0, 1.4, and 1.8% of the applied, respectively. The minor transformation products detected in seawater were CL 325,195; Unknown A; Unknown C; and Unknown D formed at maximum concentrations of 2.8, 1.3, 1.7, and 1.9% of the applied, respectively. Volatiles were not formed.

The radioactive balance was 100.7 ± 2.2%, 89.6 ± 1.4%, 102.6 ± 1.3%, and 89.0 ± 1.2% of the applied at pH 5, pH 7, pH 9, and seawater at 25EC, respectively. At test termination, the concentration of the parent compound at 25EC decreased from 93.3% at day 0 to 22.2% of the initial at pH 5, decreased from 78.4% of the initial at day 0 to not detectable by day 3 at pH 7, decreased from 52.3% of the initial at day 0 to not detectable by 24 hours at pH 9, and decreased from 58.0% of the initial at day 0 to not detectable by 24 hours in seawater. At pH 5, the major transformation product detected was CL 322,250 with a maximum concentration of 73.9% of the applied amount observed at the day 30. At pH 7, the major transformation products detected were CL 322,250 and Unknown B with maximum concentrations of 72.4% and 29.6% of the applied observed on the 7th and 30th days of incubation, respectively. At pH 9, the major transformation product detected was CL 322,250, with a maximum concentration of 96.9% of the applied amount observed on the 7th day of incubation. In seawater, the major transformation product detected was CL 322,250 with a maximum concentration of 96.3% of the applied amount observed 24 hours after incubation. The minor transformation products detected at pH 5 were CL 325,195; Unknown C; and Unknown D formed at maximum concentrations of 2.9, 2.1, and 2.3% of the applied, respectively. The minor transformation products detected at pH 7 were CL 325,195; Unknown A; Unknown C; and Unknown D formed at maximum concentration of 1.4, 7.2, 1.5, and 1.9% of the applied, respectively. The minor transformation products detected at pH 9 were CL 325,195; Unknown A; Unknown C; Unknown D; and Unknown F formed at maximum concentrations of 2.2, 1.2, 1.0, 1,9, and 1.4% of the applied, respectively. The minor transformation products detected in seawater were CL 325,195; Unknown A; Unknown C; Unknown D; and Unknown F formed at maximum concentrations of 2.7, 1.1, 1.0, 1.6, and 1.7% of the applied, respectively. Volatiles were not formed.

The hydrolytic half-lives of [^{14}C]-R107894 in pH 5, pH 7, pH 9 and seawater at 25EC were calculated as 15 days, and 8, 2, and 3 hours, respectively. The corresponding values for [^{14}C]-R107894 incubated at 10EC were 168 days, and 69, 12, and 15 hours, respectively. [^{14}C]-R107894 was found to be hydrolytically unstable under the conditions of the test. Rapid hydrolysis was observed in pH 7, pH 9, and seawater incubated at 25EC, in comparison with that observed at pH 5. While hydrolysis was slower at 10EC, [^{14}C]-R107894 would still be classified as unstable.

In the supplemental study (MRID No. 456739-09), solutions of [^{14}C]-R107894 in aqueous buffer (pH 7 and pH 9) and seawater were incubated at 10EC and 25EC for up to 96 hours to investigate the hydrolytic stability of R107894. Two hydrolysis products were detected together with two unknowns (A and B) which were only present in the pH 7 samples. The hydrolysis products (CL 322,250 and CL 325,195) were confirmed as being present in all the samples analyzed and the unknowns were identified as isomers of a condensation reaction between Tris(tris(hydroxymethyl)amino methane, from the pH 7 buffer) and CL 322,250. The

unknowns were not true hydrolysis products from the incubation, but artifacts arising from the buffer used with the pH 7 samples.

2. Photodegradation in Water (Guideline No. OPPTS 161-2, Waived)

The Agency has waived data requirements for the photodegradation of ECONEA™ Technical. The active ingredient 1H Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl-5-(trifluoromethyl) is hydrolytically unstable and rapidly degrades. Photolysis studies were, therefore, not required.

3. Anaerobic Aquatic Metabolism (Guideline No. OPPTS 162-3, MRID No. 456739-10)

This anaerobic aquatic metabolism study was reviewed by the Agency and found to be acceptable for the active ingredient 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as R107894. The anaerobic aquatic metabolism data requirements for ECONEA™ Technical have been satisfied.

The anaerobic biotransformation of [^{14}C]-R107894 was studied in both a freshwater-sediment and a marine-sediment test system from Scotland for 52 weeks in the dark at 21°C. [^{14}C]-R107894 was applied at the rate of 69 μg/L to the surface of the water in each sample. The sediment/water ratio used was 15g/150mL. The test system consisted of borosilicate glass cylinders attached with traps for the collection of CO_2 and volatile organic compounds. Samples were analysed at 0, 3, 7, 14 and 30 days and 8, 13, 17, 26, 39, and 52 weeks of incubation. Surface water was separated from the sediment by decanting and transferred into separate amberlite jars. The water samples were not extracted and the sediment samples were extracted with acetonitrile twice with approximately 50 mL. [^{14}C]-R107894 residues were analysed by thin layer chromatography (TLC) (using a silica gel 60F_{254} TLC plate and developed in toluene:acetone:methanol:acetic acid) and high performance liquid chromatography (HPLC) (using a Hewlett-Packard 1050 series). Identification of the transformation products was done by co-chromatography.

The test conditions outlined in the study protocol were maintained throughout the study. The mean total recovery of radiolabelled material after 52 weeks was 100.4±4.8% and 96.97±2.2% of the applied in the freshwater-sediment system and the marine-sediment system, respectively. The mean total recovery of radiolabelled material in the surface water and sediment of the freshwater test system was 26.30±1.1% and 22.91±0.9% of the applied amount, respectively. In the marine test system, the mean total recovery of radiolabelled material in the surface water and sediment was 57.68±0.2% and 22.46±1.2% of the applied amount, respectively.

In the fresh water test system, extractable [^{14}C]-residues in sediment decreased from a high of 62.80% at day 7 to 22.91% of the applied amount at the end of incubation period. Non-extractable [^{14}C]-residues in sediment increased from a low of 0.30% at day 3 to 50.96% of the applied amount at the end of the incubation period. In the marine test system, extractable [^{14}C]-residues in sediment decreased from a high of 32.29% at day 14 to 22.46% of the applied amount at the end of incubation period. Non-extractable [^{14}C]-residues in sediment increased from a low of 1.01% at day 3 to 16.52% of the applied amount at the end of incubation period. At the end of the study, 0.11% and 0.02% of the recovered radioactivity was present as CO_2 and volatile organic compounds, respectively, in the marine test system. In the fresh water test system, 0.04% and 0.02% of the recovered radioactivity was present as CO_2 and volatile organic compounds, respectively.

In the fresh water test system, the concentration of R107894 in surface water and sediment decreased from 90.19% at day 0 to 1.80% of the applied amount at study termination. In the marine test system, the concentration of R107894 in surface water and sediment decreased from 92.36% to 0.06% of the applied amount at study termination.

The major transformation products of both the fresh water system and the marine system detected by HPLC analysis in water and sediment were CL 322,250 and CL 325,195. Maximum and minimum concentrations in the water of the freshwater test system were 44.10% and 2.56% of the applied amount, for CL 322,250, while CL 325,195 was reported to be below the detection limit throughout the incubation period. Maximum and minimum concentrations in the water of the marine test system were 60.34% and 1.99% of the applied amount for CL 322,250, and 6.64% and below the detection limit for CL 325,195. Maximum and minimum concentrations in the sediment of the freshwater test system were 10.05% and 4.62% of the applied amount for CL 322,250, and 1.29% and 1.16% of the applied amount for CL 325,195. Maximum and minimum concentrations in the sediment of the marine test system were 16.35% and 2.38% of the applied amount, for CL 322,250, and 1.39% and 0.52% of the applied amount for CL 325,195.

The 1st order 50% decline time (DT50) for the freshwater test system was 10 days and the 90% decline time (DT90) was 113 days. For the marine test system, the 1.5 order DT50 was 0.03 days and the DT90 was 0.83 days. The rates of degradation were estimated by fitting the data to the Timmes, Frehse, and Laska model. Degradation was very rapid in the marine test system and the degradation rates of R107894 in each of the compartments could not be estimated with any degree of accuracy due to the variability in the total levels of radioactivity in each of the compartments over the incubation period.

4. Aerobic Aquatic Metabolism (Guideline No. OPPTS 162-4, MRID Nos. 456739-11 and 456739-12)

This aerobic aquatic metabolism study was reviewed by the Agency and found to be acceptable for the active ingredient 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as R107894. The aerobic aquatic metabolism data requirements for ECONEA™ Technical have been satisfied.

In the main part of the study (MRID No. 456739-11), the biotransformation of radiolabelled [^{14}C]-R107894 was studied in a freshwater/sediment system (water pH 6.5, silt loam, organic carbon 2.5%) and a marine water/sediment system (water pH 8.04, sandy loam, pH 7.53, organic carbon 0.8%) collected from Bogton Loch and Seaby Bay in Scotland. The experiment was performed for 30 days under aerobic conditions in the dark at 21^{0}C. Radiolabelled R107894 was applied at the rate of 0.5 mg/L. The test system consisted of borosilicate glass cylinders (previously silanised; 15.9 cm^2 cross-sectional area) as the incubation vessel and included a series of three traps for trapping non-specific [^{14}C]-organic volatiles and liberated $^{14}CO_2$. Samples were collected at 0, 2 hours, and 1, 3, 7, 15, and 30 days of incubation. The water samples were not extracted. The sediment samples were extracted twice with 50 ml of acetonitrile and then shaken for 1 hour, followed by centrifugation for 15 minutes. Quantification and identification of the [^{14}C]-R107894 residues was performed using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

For the silt loam (freshwater) test system, the mean overall recovery of radiolabelled material was 93.8 ± 5.2% of the applied amount. For the loamy sand (marine water) test system, the mean overall recovery of radiolabelled material was 95.5 ± 4.4% of the applied amount.

The concentration of the parent compound in freshwater immediately after the application showed a mean of 51.2% of the applied amount and had dropped below the detection limit by the end of the study period (Day 30). The concentration of the parent compound in the silt loam (freshwater) sediment decreased from a mean of 36.3% of the applied amount at Day 0 to a mean of 16.4% of the applied amount at the study termination. The concentration of the parent compound in marine water decreased from a mean of 77.2% of the applied amount at Day 0 to below the detection limit by Day 15 of the study. The concentration of the parent compound in loamy sand (marine) sediment decreased from a mean of 18.05% of the applied amount at Day 0 to a mean of 4.04% by Day 7.

The DT50 and DT90 values were estimated by visual inspection of the data by the Registrant. The DT50 for [^{14}C]-R107894 in the freshwater silt loam system was estimated as being between 3 and 7 days and the DT90 was estimated as being just over 30 days. In the marine water loamy sand test system, the DT50 and DT90 were estimated as being less than 1 day and approximately 7 days, respectively. The two major transformation products were CL 322,250 and Unknown B (a supplementary study tentatively identified this component as debrominated CL 322,250). There were four minor transformation products. These minor transformation products were referred to as CL 325,195, Unknown A, Unknown C, and Unknown D.

For the silt loam sediments, extractable ^{14}C-residues decreased from a mean of 38.1% of the applied amount at Day 0 to a mean of 26.2% of the applied amount at study termination. Non-extractable [^{14}C]-residues increased from a mean of 1.82% of the applied amount at Day 0 to a mean of 36.43% of the applied amount at the end of incubation period. For the loam sand sediments, extractable ^{14}C-residues increased from a mean of 21.4% of the applied amount at Day 0 to a mean of 33.7% of the applied amount at study termination. Non-extractable [^{14}C]-residues increased from a mean of 0.275% of the applied amount at Day 0 to a mean of 6.54% of

the applied amount at the end of the incubation period.

For the freshwater silt loam sediment system, there were no detectable levels of radioactivity present as CO_2 or volatile compounds at the end of the study. For the marine water loamy sand sediment system, a mean of 0.02% of the recovered radioactivity was present as CO_2. Volatile compounds were not detectable.

A supplemental study (MRID No. 456739-12) was also performed. One of the major transformation products from the main study (MRID 456739-11) was labeled as Unknown B and it had a retention time of approximately 26 minutes following the analysis of samples generated by the loamy sand (marine) test system. For this supplemental study, two water samples from Day 30 were taken and concentrated by solid phase extraction. The concentrated samples were analyzed by negative ion electrospray liquid chromatography mass spectrometry in addition to radiochemical detection. Two peaks were identified in the radiochromatogram during the supplementary study. The latter of these was confirmed as CL 322,250 by comparison of retention time, full scan spectrum and daughter spectrum to those obtained following the analysis of authentic CL 322,250. The first peak (Unknown B) was tentatively postulated as debrominated CL 322,250 based on comparison of retention times, spectra and daughter spectra for this peak and the CL 322,250 reference standard.

5. Adsorption/Desorption (Guideline No. OPPTS 163-1, MRID No. 456739-13)

This adsorption/desorption study was reviewed by the Agency and found acceptable for the active ingredient 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), also known as R107894. The adsorption/desorption data requirements for ECONEA™ Technical have been fulfilled.

The adsorption/desorption characteristics of [^{14}C]-R107894 were studied in two freshwater soils, sandy loam and silt loam, and two marine soils, sand and loam, from Scotland in a batch equilibrium experiment. The adsorption phase of the study was carried out by equilibrating air-dried/fresh soil with [^{14}C]-R107894 at 0, 54, 109, 268, and 518 ng/g soil for sandy loam and silt loam and at 0, 47, 96, 242, and 484 ng/g soil for sand and loam in the dark at 10 ± 2 °C for 4 hrs for all the soils but sand, which was equilibrated for 8 hrs. The equilibrating solution used was 0.01M $CaCl_2$ or seawater, with a soil/solution ratio of 2g/10g. The desorption phase of the study was carried out by adding a weight of 0.01M calcium chloride or seawater, approximately equal to that removed as supernatant, to each soil type. The tubes were shaken and analyzed as in the adsorption phase.

The supernatant solution after adsorption and desorption was separated by centrifugation. The supernatant was not extracted. [^{14}C]-R107894 residues were analysed by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). HPLC analysis was carried out using a Hewlett-Packard 1050 series HPLC equipped with an autosampler, u.v. detector and a solvent programmer, connected to an Inertsil Phenyl guard and HPLC column (1 cm and 25 cm x 4.6 mm; 5 μm; Hichrom) and a Packard Flo-One A-100 Series radioactivity monitor. Aliquots of each sample were also submitted to TLC using a silica get 60_{F254} TLC plate

and developed in toluene:acetone:methanol:acetic acid. The adsorption parameters were calculated using the Freudlich adsorption isotherm.

The stability of the test material at 10 ± 2°C in 0.01M calcium chloride and seawater was determined by HPLC. Under the test conditions, [^{14}C]-R107894 was found to be unstable. However, the study author found that these test conditions best reflect those that the test material will enter in the environment. The mass balance at the end of the adsorption phase of the study was 90.99 ± 2.1, 89.45 ± 3.4, 100.5 ± 6.9, and 103.8 ± 2.0% of the applied amount in the sandy loam, silt loam, sand, and loam soils, respectively. The mass balance at the end of desorption phase was 91.50 ± 1.1, 93.70 ± 4.9, 104.3 ± 7.6, and 99.66 ± 0.9% of the applied amount in sandy loam, silt loam, sand, and loam soils, respectively.

After 4 hr of equilibration for sandy loam, silt loam, loam and 8 hr of equilibration for sand, an average of 98.89, 98.38, 97.48, and 83.18% of the applied amount was adsorbed, respectively. Average adsorption K_d values were 450, 335, 26, and 196 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. The average adsorption K_{oc} values were 20440, 16733, 3582, and 5588 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. K_f values were 446, 349, 22, and 183 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. At the end of the desorption phase, 0.84, 0.88, 9.62, and 1.63% of the adsorbed ^{14}C was desorbed in the sandy loam, silt loam, sand, and loam soils, respectively. Average desorption K_d values were 599, 568, 40, and 299 ml/g in sandy loam, silt loam, sand, and loam soils, respectively. The average desorption K_{oc} values were 27229, 28353, 5658, and 8543 ml/g for sandy loam, silt loam, sand, and loam soils, respectively. Desorption K_d and K_{oc} values were higher than those obtained for adsorption.

6. **Bioaccumulation in Fish (Guideline No. OPPTS 165-4, Agency Estimated BCF) (No MRID Number))**

The Agency estimated the bioconcentration of the ECONEA™ Technical degradate CL 322,250 in freshwater and saltwater fish based on the log octanol/water partition coefficient. Using equations presented in the OECD TG 305 Guideline, estimated bioconcentration factors (BCFs) of 11X (pH6) and 3X (pH8) were predicted in freshwater and saltwater fish, respectively, for the bluegill sunfish.

7. **Special Leaching Study (Guideline ASTM Standard Test Method D 5108-90, MRID No. 456732-01)**

This leaching study was reviewed by the Agency and found to be acceptable for the active ingredient 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluromethyl), also known as AF028. The leaching data requirements for ECONEA™ Technical have been satisfied.

The leach rate determination of Sigma Nexxium 20 paint was studied using the ASTM D 5108-90 Method: *Standard Test Method for Organotin Release Rates of Antifouling Coating Systems in Sea Water*, specifically designed for antifoulants. The study was conducted to

determine the rate at which two active ingredients, one of which is AF028, are released from Sigma Nexxium 20 Paint. The study was conducted in synthetic seawater prepared at 25 ± 2°C, using high performance liquid chromatography (HPLC). The salinity of the synthetic seawater, was maintained between 30 and 35 ppt and a pH of 7.8 to 8.2. The study of leach rate measurement was conducted for 45 days. Cylinders were put in the holding tank (food-grade polyolefin) of 100 L capacity. Synthetic seawater was continuously pumped through the tank, an activated carbon filter and a chelating resin filter at 5L/min. Leach rates were measured by exposing the cylinders to 1500 mL of synthetic seawater and rotating the cylinders for 60 minutes at 60 ± 5 rpm. The leach rates were measured on days 1, 3, 7, 10, 14, 21, 24, 28, 31, 38, 42 and 45. Samples of the leached Sigma Nexxium 20 Antifouling paint were collected and analyzed for AFO28 by HPLC.

The pseudo steady state leach rate for AFO28 was attained in 28 days. The average leach rate of AFO28 between day 28 and 45 was 8.00 $\Phi g/cm^2$/day. The average cumulative release of AFO28 was 12.9 $\Phi g/cm^2$ through day 1 and 454 $\Phi g/cm^2$ through day 45. Sigma Nexxium 20 paint was applied to polycarbonate cylinders with measurements of 2.5 inches in diameter (cylinder length not reported). The area of paint applied on the cylinder was 200 cm^2. Film thickness was at least 0.004 inches.

8. **Additional Analyses Performed by U.S. EPA (EFED) (Power Point Presentation)**

The Agency (EFED) also performed regression analyses to estimate the half-lives in freshwater and seawater for ECONEA™ Technical (parent) and its degradate CL 322,250. The analyses were based on the information provided in the study reports submitted to the Agency to fulfill the hydrolysis (MRID Nos. 456739-08 and 456739-09), anaerobic aquatic metabolism (MRID No. 456739-10, aerobic aquatic metabolism (MRID Nos. 456739-11 and 456739-12), and adsorption/desorption (MRID No. 456739-13) data requirements for the active ingredient, 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-trifluoromethyl.

The estimated half-lives and adsorption/desorption of the parent and degradate are presented in the following tables:

Table 1. Hydrolysis Half-Lives in Freshwater and Seawater at 10 and 25°C (days)

	ECONEA (parent)	
pH	10°C	25°C
5	177	15
7	2.8	0.33
9	0.56	0.1
Seawater (pH 8)	0.7	0.1

Note: 322,250 did not degrade in the hydrolysis study at any pH or temperature

Table 2. Anaerobic Aquatic Metabolism (representing sediment)

Compound	Half-life (days	Comments
ECONEA (parent) Freshwater	29	322,250 was primarily found in the water phase. Debrominated 322,250 did not decline and was found primarily in the water phase
322,250 Freshwater	31	
ECONEA (parent) Marine	0.68	
322,250 Marine	22	

Table 3. Aerobic Aquatic Metabolism (representing water column)

Compound	Half-life (days	Comments
ECONEA (parent) Freshwater	12	322,250 was primarily found in water phase in both systems. No observed formation of debrominated 322,250 in freshwater system Debrominated 322,250 did not decline in saltwater system and was found primarily in the water phase
322,250 Freshwater	Stable	
ECONEA (parent) Marine	0.62	
322,250 Marine	288	

Table 4. Adsorption of Parent ECONEA

System	Adsorption coefficients Kf (ml/g)	Adsorption coefficients K_{oc} (ml/g)	Comments
Marine Freshwater	22-183 349-446	3143-5229 17450-20273	No correlation with clay, organic matter, or pH.

Table 5. Desorption of Parent ECONEA

System	Desorption coefficients Kf (ml/g)	Desorption coefficients K_{oc} (ml/g)	Comments
Marine Freshwater	32-236 463-480	4571-6743 21818-23150	No correlation with clay, organic matter, or pH.

Table 6. Adsorption of Degradate CL 322,250

System	Adsorption coefficients Kf (ml/g)	Adsorption coefficients K_{oc} (ml/g)	Comments
Marine Freshwater	14-119 189-357	2000-3400 8591-17850	Correlation with clay and pH.

Table 7. Desorption of Degradate CL 322,250

System	Desorption coefficients Kf (ml/g)	Desorption coefficients K_{oc} (ml/g)	Comments
Marine Freshwater	30-283 1260-1685	4310-8084 57256-84250	Correlation with clay and pH.

The Agency concluded that:

- Parent degrades to 322,250 (parent minus fluorines and remaining carbon hydrated)
- 322,250 further degrades by losing a Bromine (debrominated 322,250)
- Debrominated 322,250 is only formed under anaerobic conditions or in saltwater
- Metabolism studies show 322,250 and debrominated 322,250 to be primarily in water phase
- However, mobility data on 322,250 show partitioning to sediment
- No mobility data for debrominated 322,250.

Data Gap: See Table below.

Environmental Fate Data Requirements for Econea™ Technical			
OPP Guideline	**Data Requirement**	**MRID No.**	**Data Requirement Status**
161-1	Hydrolysis	456739-08 456739-09	Satisfied
161-2	Photodegradation in Water	None	Waived
162-3	Anaerobic Aquatic Metabolism	456739-10	Satisfied
162-4	Aerobic Aquatic Metabolism	456739-11 456739-12	Satisfied
163-1	Adsorption/Desorption	456739-13	Satisfied
OECD 305	Bioaccumulation in Fish	None	Estimated
ASTM D5108-90	Special Leaching Study	476732-01	Satisfied

BIBLIOGRAPHY

MRID	CITATION
456732-01	Sinning, D.J. (2002) Leach Rate Determination of Sigma Nexxium 20 Paint Containing Sea Nine™ 211 and AF028 Antifoulings. Unpublished study prepared by Case Consulting Laboratories, Inc., New Jersey.
456739-08	Mackie, J.A. (1997) Determination of the Hydrolytic Stability of [^{14}C]-R107894: Report Number 15348. Unpublished study prepared by Inveresk Research, Scotland.
456739-09	Milligan, F.M.; Williams, S.G.P.; McGuire, G.M. (1997) Identification of Hydroytic Degradation Products of [^{14}C]-R107894: Report Number 15365: Supplement to MRID 456739-08. Unpublished study prepared by Inveresk Research, Scotland.
456739-10	Mackie, J.A. (1999) The Anaerobic Degradation of [^{14}C]-R107894 in Two Water/Sediment Systems: Report Number 17832. Unpublished study prepared by Inveresk Research, Scotland.
456739-11	Mackie, J.A. (1999) The Aerobic Degradation of [^{14}C]-R107894 in Two Water/Sediment Systems: Report Number 16787. Unpublished study prepared by Inveresk Research, Scotland.
456739-12	Unknown author(s) (1999) Identification of Unknown Component Present in a Day 30 Surface Water Following Application of [^{14}C]-R107894 to Loamy Sand Sediment: Report Number 17802: Supplement to MRID 456739-11. Unpublished study prepared by Inveresk Research, Scotland.
456739-13	Mackie, J.A. (1998) Adsorption/Desorption of [^{14}C]-R107894 in Sediments: Report Number 390723. Unpublished study prepared by Inveresk Research, Scotland.
None	U.S. EPA (2004) ECONEA Fate and Transport Properties. Power Point presentation presented April 13, 2004, by Jim Breithaupt, Environmental Fate and Effects Division (EFED).

DATA PACKAGE BEAN SHEET

Decision #: 220066

DP #: (330789)

Date: 20-Oct-2006

* * * Registration Information * * *

Registration: 43813-ET - ECONEA TECHNICAL

Company: 43813 - JANSSEN PHARMACEUTICA INC.

Risk Manager: RM 33 - Marshall Swindell - (703) 308-8341 Room# PY1 S-8828

Risk Manager Reviewer: Karen Leavy KLEAVY

Sent Date: 31-May-2006 Calculated Due Date: 08-Jan-2007 Edited Due Date:

Type of Registration: Product Registration - Section 3

Action Desc: (A41) NEW AI;NON-FODD USE;OUTDOOR;OTHER USES;

Ingredients: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-(93.2%)

* * * Data Package Information * * *

Expedite: Yes ● No Date Sent: 11-Jul-2006 Due Back:

DP Ingredient: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-

DP Title:

CSF Included: Yes ● No Label Included: Yes ● No Parent DP #:

Assigned To	Date In	Date Out	
Organization: AD / RASSB	01-Aug-2006	24-Aug-2006	Last Possible Science Due Date: 03-Jul-2006
Team Name: RASSB1	01-Aug-2006	24-Aug-2006	Science Due Date:
Reviewer Name: Gowda, Srinivas	01-Aug-2006	24-Aug-2006	Sub Data Package Due Date:
Contractor Name:			

* * * Studies Sent for Review * * *

No Studies

* * * Additional Data Package for this Decision * * *

Printed on Page 2

* * * Data Package Instructions * * *

Review and Create an Environmental Fate Science Chapter for ECONEA Technical
PRIA, Action Code A41.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES,
AND TOXIC SUBSTANCES

November 15, 2006

MEMORANDUM

SUBJECT: Occupational Exposure Assessment of ***Econea***: A New Active Ingredient, *1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)*, Proposed as an Antifoulant in the Manufacturing-Use Product (MUP) **ECONEA™ Technical** (EPA File Symbol 43813-ET, 93.2% a.i.), and in the Formulated End-Use Product (EP) **Sigma Nexxium 20 Antifouling** (EPA File Symbol 11350-GL, 3.4% a.i..).

TO: Dennis Edwards, Chief
Marshall Swindell, Product Manager, Team 33
Regulatory Management Branch I
Antimicrobials Division (7510P)

FROM: Doreen Aviado, Biologist Doreen Aviado 11/15/06
Team Two
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

THRU: Nader Elkassabany, Team Leader Wcwk for 11/15/06
Team Two
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Norm Cook, Chief 11/15/06
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

DP Barcodes: D295928, D327535, D327536 & D330452

Pesticide Chemical/No.: MUP: 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl) (AC 303268, R107894, CL 303268, AF028) *Econea* / 119093.
EP: *Econea* /119093 and *Sea-Nine 211* (C-9211, RH-287, or Kathon 287T) / 128101

Registrants: Janssen Pharmaceutica Inc. (MUP); and Sigma Coatings USA (EP)

EPA File Symbols: 43813-ET: *ECONEA™ Technical* (MUP)
11350-GL: *Sigma Nexxium 20 Antifouling* (EP)

MRID No.: 468466-02

EXECUTIVE SUMMARY

The Antimicrobials Division (AD), Product Management Team 33, requested that the Risk Assessment and Science Support Branch (RASSB) conduct an occupational exposure assessment for a proposed ready-to-use antifoulant paint product, ***Sigma Nexxium 20 Antifouling***, containing a new antifoulant active ingredient, *1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)* (a.k.a., *Econea*). The active ingredient *Econea* is a degradate of an agricultural use insecticide-miticide (Chlorfenapyr, PC Code 129093) which is registered primarily for foliar applications to greenhouse grown ornamentals and fruiting vegetables. Chlorfenapyr as the precursor for *Econea* is metabolized to form this new active.

Econea is slated for registration as a technical grade active (93.2% a.i.) in Janssen Pharmaceutica's formulator source product ***ECONEA™ Technical*** (EPA File Symbol 43813-ET); intended solely as a manufacturing-use product (MUP) in formulating antifoulant paints.[1] Each formulator using this MUP is responsible for obtaining EPA registration for their pesticide end-use product (EP). At present, the one proposed formulated end-use product (EP) under AD/RASSB exposure review is Sigma Coatings' ***Sigma Nexxium 20 Antifouling*** (EPA File Symbol 11350-GL), a self-polishing antifouling coating for commercial application to underwater hulls of boats (i.e., pleasure, military and commercial vessels) in fresh water and marine environments.[2] This EP is for use in commercial or government shipyards only and contains a co-biocide mixture of 3.4% *Econea* a.i., and 3.4% *Sea-Nine 211* a.i. (C-9211, RH-287, or Kathon 287T). *Econea* will control the growth of barnacles and other hard fouling organisms and *Sea-Nine 211* is added to control the growth of algae. Since *Sea-Nine 211* is already registered with the Agency for similar antifoulant use patterns (PC Code 128101) only the new active *Econea* was evaluated in a screening-level assessment for occupational exposure concerns.

Chemical-specific worker exposure monitoring data were not submitted, or cited, for use in assessing exposures for industrial shipyard workers as painters and handlers of ***Sigma***

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[1] Formulator source products, such as *ECONEA™ Technical*, which are used as the technical active to manufacture pesticide end products are typically not assessed for human exposure concerns as part of the registration process. The Agency assumes that occupational workplace safety standards set by the Occupational Safety and Health Administration (OSHA) for industrial manufacturing facilities and any specified personal protective equipment (PPE) on the MUP label (or chemical MSDS) are adequate to protect workers in contact with such chemicals/source products during formulation. Also, the Agency does not impose human exposure data requirements (*Series 875 Data Guidelines*) for MUPs, only for typical end-use products (EPs) which address handler/postapplication exposure.

[2] In 2004 *Sigma Coatings USA* applied for an experimental use permit (EUP) for a *Sigma Nexxium 710 Antifouling* paint formulation containing 3.25% *Econea* and 3.26% Sea-Nine 211. A preliminary screening-level human exposure assessment was conducted at that time with no risk concern outcomes (AD/RASSB Review Memorandum for D310254 by C. Walls, Chemist, dated November 23, 2004). AD recently received a registration application for an additional proposed *Econea*-based antifoulant EP as of September, 2006. The EP is TRILUX 44-White (EPA File Symbol 2693-EEN) from International Paint LLC containing a co-biocide mixture of 3.9% Econea and 4.12% Zinc pyrithione (PC Code 088002). This product has been submitted for RASSB review of leach rate study data (October, 2006), but not for a worker exposure assessment.

Nexxium 20 Antifouling paint. However, certain data were provided by Janssen Pharmaceutica, Inc. in the form of an occupational exposure report (the most recent version as MRID 468466-02) dated May 15, 2006, entitled "*Revision 1 to MRID No. 46751303: Screening Level Occupational Exposure Assessments For R107894 (AC or CL 303268) As An Antifoulant In Paint Applied To Underwater Hulls of Pleasure, Military, and Commercial Craft.*" This report supports the use pattern for the MUP, but is specifically intended to satisfy human exposure data needs for the formulated ***Sigma Nexxium 20 Antifouling*** EP and addresses potential occupational exposure concerns for representative use patterns. The submitted assessments are intended to qualitatively evaluate the potential worker exposures during shipyard painting operations and address, in a broad sense, the Human Exposure Data requirements under Series 875 Guidelines.[3] As a conservative screening tool the report also includes quantitative dermal/inhalation exposure estimates and calculated MOEs for different painter scenarios (i.e., paint mixer/loader/applicator scenarios) based on surrogate data from the Pesticide Handlers Exposure Database (PHED).

An evaluation of non-dietary occupational exposures was conducted by AD/RASSB on representative scenarios for exposure routes of concern based on the product use patterns and the toxicity of the active ingredient. The assessment relied on certain Agency standard values and surrogate data sources to develop scenarios; including surrogate unit exposure data taken from PHED, Version 1.1 (U.S. EPA, 1998). Registrant-submitted information on shipyard worker observations (MRID 468466-02) was used as "Product Use" and "Description of Human Activity" data to aid in calculating handler doses.

Endpoints for this risk assessment were obtained from the AD/RASSB toxicology memorandum written in support of the registration action for the technical source MUP product ***ECONEA™ Technical*** (U.S. EPA, 2006). Based on submitted acute toxicity studies, *Econea* (93.2% a.i., MUP) is most acutely toxic via the oral (Toxicity Category I - DANGER) and inhalation (Toxicity Category II) routes and the MUP concentration does not elicit dermal sensitization. In the case of the formulated ***Sigma Nexxium 20 Antifouling*** paint, the EP product appears to be of moderate toxicity for eye irritation (Toxicity Category II) and acute oral and primary skin irritation (Toxicity Category III). Study data indicate that the formulation is a dermal sensitizer. The ***Sigma Nexxium 20 Antifouling*** draft product labeling includes the signal word "CAUTION" and precautionary statements for handlers to wear at a minimum, a powered air purifying respirator (PAPR) and indirect references to other personal protective equipment (PPE) in the form of gloves and protective clothing.

[3] The data covered in the report covers the minimum requirements for an EP (i.e., data to address GLN 875.1700 and 875.2700 *Product Use Information*, and GLN 875.2800 *Description of Human Activity* to better characterize the nature of the potential application/post-application exposures). The quantitative exposure estimates in the report follow the Agency's initial recommendation from a March 7, 2001 pre-registration meeting, to submit a 'human health exposure risk assessment' as an alternative to generating any Application and/or Post-Application Guideline Studies under GLN 875.1100/GLN 875.1200 *Dermal Exposure Outdoor/Indoor* and GLN 875.1300/GLN 875.1400 *Inhalation Exposure Outdoor/Indoor*.

The short-term (ST)/intermediate-term (IT) dermal endpoint NOAEL for *Econea* is <6.3 mg/kg/day taken from a 90-day oral toxicity study in the rat. The NOAEL is derived from the LOAEL observed for females. A 90-day inhalation toxicity study in the rat was used for the ST/IT inhalation endpoint. The NOAEL could not be calculated so the LOAEL air concentration of <20mg/m^3 was selected based on local irritation effects of the dorsal region of the nose. Since the adverse effects were due to point-of-entry localized irritation, it was assumed that an inhalation reference dose (RfD) could be estimated as 5.7 mg/kg/day if needed as an alternate to the air concentration LOAEL in developing exposure scenario risk determinations, as per Agency methodologies (U.S. EPA, 1989 and 1994).

The level of concern (LOC) for *Econea* dermal and inhalation route exposures is 300 [i.e., a margin of exposure (MOE) less than 300 indicates potential risk concerns] for occupational scenarios. This LOC is based on uncertainty factors of 10x interspecies extrapolation, 10x intraspecies variation, and 3x for data gaps where a LOAEL was selected for lack of a NOAEL. A default of 100% absorption was applied for the dermal route as route-to-route extrapolation from the oral toxicity endpoint. An absorption factor was not applied to inhalation exposures since the endpoint was derived from an inhalation route-specific study. A body weight of an average male adult (i.e., 70 kg) was used to estimate exposure doses.

Occupational Exposure Summary

Occupational handler risks were assessed for shipyard workers in contact with antifoulant paint containing *Econea*. All occupational exposure scenarios were assumed to be short-term (ST), 1 to 30 days, and intermediate-term (IT), occurring for periods of 30 days to 6 months. No endpoints were identified for long-term (LT) exposure scenarios (i.e., exceeding 6 months duration). Handler dermal and inhalation exposure scenarios were identified for shipyard workers involved with painting operations using ***Sigma Nexxium 20 Antifouling***; including paint tenders who perform ancillary tasks of mixing/loading paints, and paint applicators engaged in predominantly airless sprayer applications, and to a minor degree, brush/roller touch-up painting.

The handler exposures were estimated at baseline (i.e., single layer clothing, no gloves or respirator) and for various personal protective equipment (PPE) scenarios where different clothing attire (i.e., gloves and Tyvek coveralls) and respirator options (i.e., dust/mist, organic/vapour, powered air and supplied air respirators) are employed, as applicable. The Agency generally is not concerned with a calculated MOE greater than or equal to the target MOE (i.e., level of concern). Results from the screening-level occupational exposure assessment indicate that the following handler scenarios exceed the Agency's level of concern (i.e., MOEs below the target of 300) and therefore pose potential risks:

Industrial Shipyard Scenarios:

Paint Tenders (Mixer/Loader- open pour):
ST/IT dermal exposure: Baseline MOE = 9

Paint Applicators (Airless Sprayer- primary):

ST/IT dermal exposure:	Baseline MOE = 2;
	PPE-Gloves MOE = 5;
	PPE-Tyvek Coveralls MOE = 5.
ST/IT inhalation exposure:	Baseline MOE = 7;
	PPE-PHED Organic Vapour Respirator MOE = 60.

Paint Applicators (Brush/Roller- secondary):

ST/IT dermal exposure:	Baseline MOE = 1
	PPE-Gloves MOE = 9;
	PPE-Tyvek Coveralls MOE = 9.

All other scenarios evaluated yielded exposure estimates which do not pose a risk concern (i.e., MOEs well above the 300 target). The handler assessment confirms the need for clear labeling language requiring dermal and respiratory PPE during product use and adherence of industrial safety standards for workers engaged in commercial antifoulant applications/handling.

Occupational postapplication exposures were not assessed. No exposure data have been submitted to the Agency to determine the extent of postapplication exposures for worker scenarios. It is assumed that exposures in industrial settings following painting operations will be less than handler exposure during painting. Inhalation concerns post-treatment should be minimal due to the non-volatile nature of the active ingredient (1.9×10^{-8} kPa at 20°C) and the lack of aerosol/spray mist generation once boat hull painting is done. *Econea* is unlikely to volatilize appreciably at room temperature during clean-up tasks. Dermal contact with painted surfaces is also not a concern since workers do not remain in work areas or re-enter as fresh paint is drying.

Data Limitations and Uncertainties

The following items are some of the data limitations and uncertainties associated with the occupational exposure assessment:

- Chemical-specific exposure monitoring data were not available; therefore as is policy, the Agency used surrogate data sources and standard approaches which may not realistically estimate exposure during actual use conditions. Surrogate unit exposure values were taken from the Pesticide Handlers Exposure Database (PHED) (USEPA, 1998). (See Appendix A for a summary of PHED data).

- AD/RASSB relied upon professional judgment of industrial practices, product labeling use rates/methods and certain registrant-provided inputs (taken from submitted data MRID 468466-02) for estimating quantity handled/day and surface area painted/day. It is not known if the observational data for shipyard painting operations (MRID 468466-02) may actually underestimate the quantity handled/area painted (e.g., application rate of 500 sq ft/hr and 3000 sq ft/day painted surface area).

- The dermal-route exposure risks were based on a toxicological endpoint from a 90-day oral toxicity study in the rat (i.e., the NOAEL is <6.3 mg/kg/day). It should be noted that the registrant had conducted a 90-day dermal study in the rat (MRID 46802201) which was deficient for lack of performed lung histopathology, but upgradeable. The available study data indicates a dermal NOAEL of 100 mg/kg/day and a systemic NOAEL of 300 mg/kg/day. These data can be upgraded if lung pathology is evaluated to note the frequency of lung lesions at the low and intermediate dose levels to better define the NOAEL/LOAEL. If the dermal study is used for toxicological hazard, and the 3X database uncertainty factor removed, then several occupational scenarios which triggered dermal risk concerns will yield more favorable outcomes.

Review Outcome and Recommendations

At this time the screening-level assessment supports a "conditional" registration of the new active ingredient, *1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)* (a.k.a., *Econea*) and its intended commercial antifoulant use patterns, with the following considerations:

ECONEA™ Technical MUP 93.2% a.i. (EPA File Symbol 43813-ET):

- As a note to PM Team 33 regulatory management, the ***ECONEA™ Technical*** MUP draft product labeling of May 2, 2002 is incomplete. Specific formulator use directions covering the industrial application methods/use rates were not cited on the label nor provided in the form of a technical bulletin.

- Also, precautionary labeling statements for this powder formulation will need revising according to FIFRA guidance for Toxicity Category I-II products which carry the "DANGER" Signal Word. Specifically, the addition (beyond the cited protective eyewear, e.g., goggles, face shield or safety glasses) of clear PPE statements for handlers to wear long-sleeved shirt, long pants, socks and shoes, chemical-resistant gloves, and a dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C).

Sigma Nexxium 20 Antifouling EP 3.4% a.i. (EPA File Symbol 11350-GL):

- The PPE precautionary labeling statements are not acceptable on the draft labeling/technical bulletin submitted May 7, 2002 for the ***Sigma Nexxium 20 Antifouling*** EP. Based on acute toxicity (Categories II-III) and primary use in airless spray applications, appropriate PPE must include clothing and respiratory protection reflective of industry standards for commercial handlers of antifoulant paints. Specifically, PPE shall include use of protective eyewear (e.g., goggles, face shield or safety glasses), statements for handlers to wear long-sleeved shirt, long pants, socks and shoes, and chemical-resistant gloves. The labeling already states that *"While spraying and/or sanding boat surfaces, wear a minimum of a powered air purifying respirator (PAPR) jointly approved by the Mining Enforcement and Safety Administration and the National Institute for Occupational Safety and Health."* Due to concerns for spray paint operators

in confined/enclosed spaces, the respiratory protection statement can be revised to include *"...wear a supplied-air respirator (SAR) or powered air purifying respirator (PAPR)..."*.

- Since the EP is a dermal sensitizer, the labeling must state: *Prolonged or Frequently Repeated Skin Contact May Cause Allergic Reactions in Some Individuals.* Additional dermal PPE statements should be considered as: *Wear a Tyvek hooded coverall or the exact equivalent, impervious gloves and impervious footwear that protects the lower legs.* The precautionary labeling should also include: *Unprotected persons should be kept out of the treatment areas within walking distance of XXX feet* (i.e., registrant-specified distance, such as 100 feet).

- The EP labeling includes certain restrictions for commercial shipyard use only. However, the PM Team 33 may wish to require additional, prominent, use restriction statements such as: *For Professional Application Only, Not for Use by Private Applicators or Marinas.*

- The occupational assessment covers use patterns specified for the formulated EP, ***Sigma Nexxium 20 Antifouling*** as a shipyard antifoulant coating. If this EP use pattern is amended to include uses outside of commercial shipyards, for smaller craft in marinas or D-I-Y applications, AD/RASSB will need to conduct a revised assessment of potential risks.

1.0 INTRODUCTION

1.1 Purpose

In support of registration for ***ECONEA ™ Technical*** MUP, as the a.i. technical source product, and the formulated ***Sigma Nexxium 20 Antifouling*** paint EP, *Janssen Pharmaceutica Inc.* (MUP registrant) and *Sigma Coatings USA* (EP registrant) jointly submitted registration applications to the Agency in May, 2002. The Antimicrobials Division (AD), Product Management Team 33, requested that the Risk Assessment and Science Support Branch (RASSB) review the registration application for the new antifoulant active ingredient, *1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)* (a.k.a. *Econeo)* in its proposed use in the formulated end-use product (EP) ***Sigma Nexxium 20 Antifouling***, a self-polishing antifouling coating containing a co-biocide mixture of 3.4% *Econeo* a.i., and 3.4% *Seo-Nine 211* a.i. (C-9211, RH-287, or Kathon 287T). As part of the review AD/RASSB conducted an occupational exposure assessment of the EP, as detailed in this memorandum.

1.2 Criteria for Conducting Exposure Assessments

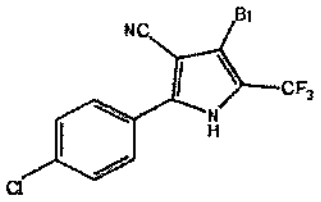
An occupational exposure assessment is required for an active ingredient if (1) certain toxicological criteria are triggered and (2) there is potential exposure to handlers (mixers, loaders, applicators, etc.) during use or to persons entering treated sites after application is complete. For *Econeo*, both criteria are met in the proposed use as a commercial antifoulant coating.

As part of this review of ***Sigma Nexxium 20 Antifouling***, an exposure assessment was conducted to determine if there are any non-dietary occupational exposure concerns for adult handlers during mixing/loading/application of antifoulant coatings in shipyards and any workplace postapplication concerns. The Agency reviewed the draft product labeling and technical data sheet, submitted screening-level assessment (MRID 468466-02), and the toxicity profile/hazard characterization for this compound, in order to assess occupational exposures.

1.3 Chemical Identification and Physical/Chemical Properties

Econea is a metabolite (AC 303268 or CL 303268) of the registered insecticide *Chlorfenapyr* (PC Code 129093). It functions as an uncoupler of oxidative phosphorylation in the mitochondria of cells as the mode of action. The physical/chemical characteristics of *Econea* are identified in Tables 1 and 2 as follows:

Table 1. Chemical Nomenclature/Product Identification for *Econea* Technical Grade Active

Compound	The chemical structure of AC 303268 is as follows:  *Econea*
Common name	*Econea* known as AC 303268 (trade name), R107894, Ecollea 028 or AF028.
PC Code	119093
IUPAC name*	1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)
CAS #	122454-29-9
Empirical Formula	$C_{12}H_5BrClF_3N_2$
MUP/EP formulations	Formulated as *ECONEA ™ Technical* (MUP containing 93.2% AC 303268) and *Sigma Nexxium 20 Antifouling* (EP containing 3.4% AC 303268 and 3.4% Sea-Nine 211 as co-biocides)

Source: Table based on AD/RASSB Product Chemistry Review for AC 303268 TGAI, by R. Quick, Chemist, dated August 27, 2003, (D289033); also, an AD/RASSB Environmental Fate Assessment of Econea Technical by S. Guwda, Chemist, dated August 17, 2006 (D330789). * International Union of Pure and Applied Chemistry designation (IUPAC).

Table 2. Physicochemical Properties of *Econea* Technical Grade Active	
Parameter	**Value**
Physical state	TGAI is a powder
Color	Off-white to pale yellow-brown
Odor	Slightly sweet, marzipan-like
Melting point/range	252.3 - 253.4 °C
pH	5.16 at 22°C (0.1% w/v dispersion in water)
Relative density	1.714 g/ml (s.d. 0.007)
Water solubility (mg/L at 20°C)	solvent solubility freshwater (pH 4.9) 0.17 mg/L (Low Water Solubility) seawater (pH 8.1) 0.16 mg/L
Vapor pressure at 25°C	1.9×10^{-8} kPa at 20°C and 4.6×10^{-8} kPa at 25°C (non-volatile)
Dissociation constant (pK_a)	$pK_a = 7.08$ at 26°C
Octanol/water partition coefficient Log(K_{ow})	log $P_{ow} = 3.5$ at pH 5
UV/visible absorption spectrum	Wavelengths of maximum absorbance are 281.4, 281.9, and 223.9 for acidic, neutral and alkaline conditions, respectively.

Source: Table based on AD/RASSB Product Chemistry Review for AC 303268 TGAI, by R. Quick, Chemist, dated August 27, 2003, (D289033).

1.4 Use Profile

The ***Sigma Nexxium 20 Antifouling*** EP is for commercial application (in commercial or government shipyards only) to metal and fiberglass boat hulls to control barnacle growth in fresh and salt water. The antifoulant coating is to be applied primarily by airless sprayer, yet a brush or roller may be used for touch-up and repair only. By restricting the use applications to commercial handlers in shipyards it is inferred that this EP will not be sold for use by either residential do-it-yourself (DIY) applicators on recreational boats, or private operators in marinas and small boatyards.

In a pre-registration meeting of March 7, 2001, the registrants (*Janssen Pharmaceutica Inc.* and *Sigma Coatings USA)* noted that the new active ingredient has been in use as an antifoulant in parts of Europe since 2000 (i.e., in Italy, Greece and Spain). They emphasized use of the EP for coating commercial vessels and government and Navy ships; and they noted that use on pleasure craft is not intended at present but may be considered by Sigma at a future date. An overview of the product use applications is shown in Table 3.

Table 3. *Sigma Nexxium 20 Antifouling* Use Overview: Proposed Use Applications Based on Draft Product Labeling/Technical Data Sheet	
Formulation	A Ready-to-Use (RTU) Viscous Self-Polishing Antifouling Paint containing 3.4% *Econea* and 3.4% *Sea-Nine 211* as co-biocides. (Net Contents: 5 U.S. Gallons). Color: Redbrown (5299)- flat
Active Ingredient % (PC Code)	*Econea* (AC 303268) 3.4% (119093)
Product Density	12.5 lbs/gal mass density (0.425 lbs a.i./gallon). Solids content 55% by volume.
Labeling Restrictions/Precautions	Applicators are Commercial/Professional Handlers – (NOT private operators or D-I-Y applicators. NOT for application by homeowners.) *Commercial Uses:* "For Commercial Use Only." "For Use in Commercial or Government Shipyards Only".
Product Use/Application Rates/Methods	*Product Use Directions:* "For use on metal and fiberglass boat hulls to control barnacle growth in fresh and salt water. Do not apply below 36°F unless care is taken to insure absence of frost/ice. Surfaces to be painted shall be free of dirt, oil, grease and other surface contaminants. Apply in accordance with Sigma's directions or specifications given in the Technical Data Sheet for 5299." *Application Rates/Methods:* "Airless Spray" painting equipment is recommended for *Sigma Nexxium 20 Antifouling* application (2100-2500 p.s.i. specified nozzle pressure; 0.021 -0.027 inch nozzle orifice). "Brush/Roller" may be used for touch-up and repair only. • Paint may be thinned with a paint thinner (max 2% in volume). • Paint should be stirred well before use, preferably by means of a mechanical mixer, to ensure homogeneity. • Theoretical Spreading Rate: 220 ft ^{2}/gal at 4 mils. dry film thickness as optimal (range: 3 mils/294 ft ^{2}/gal to 6 mils/147 ft ^{2}/gal). • Minimum Drying Times: *Touch dry* - after 1 hour; *Overcoating interval* – minimum 8 hours; *Reflloating time* – minimum 12 hours.
Personal Protective Equipment (PPE) and Precautionary Statements	*Signal Word:* CAUTION. *Warning:* Keep out of Reach of Children. *Precautionary Statements:* "Harmful if inhaled. Causes moderate eye irritation. Avoid contact with eyes or clothing. Avoid breathing spray mist. Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove contaminated clothing and wash clothing before reuse." *PPE for Applicators and other Handlers:* • (As per Draft Label): "While spraying and/or sanding boat surfaces, wear a minimum of a powered air purifying respirator (PAPR) jointly approved by the Mining Enforcement and Safety Administration and the National Institute for Occupational Safety and Health." • (As per Technical Data Sheet 5299): "Gloves and fresh air mask recommended, see safety sheets 1430, 1431 and relevant material safety data sheet."

Source: *Sigma Nexxium 20 Antifouling* draft product labeling and technical data sheet (no. 5299) dated May 7, 2002.

2.0 TOXICOLOGICAL HAZARD AND ENDPOINT SELECTION

Endpoints for the risk assessment were obtained from the AD/RASSB toxicology memorandum written in support of this registration action; specifically, the June 29, 2006 document by Toxicologists S.L. Malish and J. Chen, entitled "*Toxicity Study Review of Econea-Technical and Toxicology Endpoints for Risk Assessment*" (U.S. EPA, 2006). The review summarized the acute toxicity and subchronic studies for the technical active and selected the toxicological endpoints for assessing occupational/residential exposure risks.[4] An overview is presented in Tables 4 and 6.

2.1 Acute Toxicity

Based upon acceptable guideline studies reviewed for the technical source MUP, ***ECONEA ™ Technical*** (93.2% a.i. powder), *Econea* shows severe acute toxicity via the oral route (Toxicity Category I - DANGER) and is moderately toxic via inhalation (Toxicity Category II). It is mildly toxic through the dermal route, and from primary eye/skin irritation studies (Toxicity Categories III and IV). The MUP concentration does not elicit dermal sensitization.

In the case of the formulated ***Sigma Nexxium 20 Antifouling*** paint, the EP product appears to be of moderate toxicity (Toxicity Category II – eye irritation). It is moderately toxic for acute oral and primary skin irritation (Toxicity Category III) and mildly toxic via the dermal route (Toxicity Category IV). However, study data indicate that the formulation is a dermal sensitizer. The registrant requested a waiver for the acute inhalation data requirement based on the rationale that the active is not respirable due to the particle size distribution (5-10 microns), but the Agency denied the data waiver request since particles of that size range are still inhalable. It is not known if the registrant has further addressed this data gap. The acute toxicity data for *Econea* MUP and EP are summarized below in Tables 4 and 5.

[4] The inclusion in the toxicity study review of residential endpoints is based on any non-dietary incidental oral ingestion scenarios that may be developed for this active ingredient. Since *Econea* is an antifoulant for paints used in fresh water, the possibility of incidental oral ingestion of residues in potable drinking water might occur.

Table 4. Acute Toxicity Data on *ECONEA™ Technical* (93.2% a.i. powder) MUP				
Guideline No.	**Study Type**	**MRID #**	**Results**	**Toxicity Category**
870.1100	Acute Oral – Rat (Limit Test) **	45673915	LD_{50} Not established *[Acceptable/Guideline]*	I
870.1200	Acute Dermal - Rabbit**	45673916	LD_{50} > 2000 mg/kg for both males and females *[Acceptable/Guideline]*	III
870.1300	Acute Inhalation – Rat	45673917**	LC_{50} = 790 mg/m^3 (males) LC_{50} = 790 mg/m^3 (females) LC_{50} = 770 mg/m^3 (combined) *[Unacceptable/Guideline]* based on the fact that the percentage of particles below 4 um at the low and mid-dose level did not meet the EPA recommendations.	III
		46846601*	LC_{50} = <510 mg/ m^3 (males) LC_{50} = <510 mg/ m^3 (females) LC_{50} = <510 mg/ m^3 (combined) *[Acceptable/Guideline]*	II
870.2400	Primary Eye Irritation – Rabbit (Limit Test)**	46539401	Mildly irritating *[Acceptable/Guideline]*	III
870.2500	Primary Skin Irritation – Rabbit (Limit Test)**	45673918	Mildly irritating based on very slight erythema, but no edema at 72 hours *[Acceptable/Guideline]*	IV
870.2600	Dermal Sensitization - Guinea Pig**	45673919	Not a dermal sensitizer *[Acceptable/Guideline]*	N/A

* memorandum of June 29, 2006 from S.L. Malish and J. Chen to N. Cook (Toxicity Review of *Econea* Technical and Toxicology Endpoints for Risk Assessment) for D323129, D327538, D328778, and D330458.
** memorandum of Jan 31, 2006 from McMahon to Swindell (1H-Pyrrole…[ECONEA technical]: Review of Toxicology data submitted in Support of Registration).

Table 5. Acute Toxicity Data on *Sigma Nexxium 20 Antifouling* (3.4% a.i.) paint EP				
Guideline No.	**Study Type**	**MRID #**	**Results**	**Toxicity Category**
870.1100	Acute Oral – Rat	45673202	LD_{50} = 5068 mg/kg (males) (95% CI: 4850 – 5303) LD_{50} = 3869 mg/kg (females) (95% CI: 3754 -3987) *[Acceptable/Guideline]*	III
870.1200	Acute Dermal - Rabbit	45673203	LD_{50} = > 5050 mg/kg (males) LD_{50} = > 5050 mg/kg (females) LD_{50} = > 5050 mg/kg (combined) *[Acceptable/Guideline]*	IV
870.1300	Acute Inhalation - Rat		Data Gap - No Data *[Waiver Denied]* *	Unknown
870.2400	Primary Eye Irritation - Rabbit	45673204	Moderate irritation based on corneal opacity, erythema, and chemosis. *[Acceptable/Guideline]*	II
870.2500	Primary Skin Irritation - Rabbit	45673205	Based on observations of erythema (resolved within 7 days) and edema (resolved within 48 hours). *[Acceptable/Guideline]*	III
870.2600	Dermal Sensitization - Guinea Pig	45673206	Modified Buehler method *[Acceptable/Guideline]*	Dermal Sensitizer

Source: Table based on AD/PSB Acute toxicity Review for EPA Reg. No. 11350-GL (*Sigma Nexxium 20 Antifouling)* by C. Jiang, Chemist, dated May 18, 2005 (D316716). * Waiver for acute inhalation is denied due to particle size (5-10 microns) considered inhalable.

2.2 Dose Response Assessment

Based on the toxicity review, study data for ***ECONEA™ Technical*** (93.2% a.i.) indicate that *Econea* is not mutagenic, nor a developmental toxicant. However, study data show that neurotoxic effects in rats were seen (decreased motor activity in males, and axonal degeneration in the peroneal nerve in females). It is unknown if *Econea* is associated with reproductive or carcinogenic effects since toxicity studies to address these concerns have not been submitted to support the technical active or product registrations.

The short-term (ST)/intermediate-term (IT) dermal endpoint NOAEL for *Econea* is <6.3 mg/kg/day taken from a 90-day oral toxicity study in the rat. The NOAEL is derived from the LOAEL observed for females. A default of 100% absorption was applied for the dermal route as route-to-route extrapolation from the oral toxicity endpoint. [It should be noted that the

registrant had conducted a 90-day dermal study in the rat (MRID 46802201) which was deficient for lack of performed lung histopathology, but upgradeable. The study data indicates a dermal NOAEL of 100 mg/kg/day and a systemic NOAEL of 300 mg/kg/day. These data can be upgraded if lung pathology is evaluated to note the frequency of lung lesions at the low and intermediate dose levels to better define the NOAEL/LOAEL.]

A 90-day inhalation toxicity study in the rat was used for the ST/IT inhalation endpoint. The NOAEL could not be calculated so the LOAEL air concentration of <20mg/m^3 was selected based on local irritation effects of the dorsal region of the nose. An absorption factor was not applied to inhalation exposures because the endpoint was derived from an inhalation route-specific study. Since the adverse effects were due to point-of-entry localized irritation, it was assumed that an inhalation reference dose (RfD) could be estimated as 5.7 mg/kg/day if needed as an alternate to the air concentration LOAEL in developing exposure scenario risk determinations, as per Agency methodologies (U.S. EPA, 1989 and 1994).

The level of concern (LOC) for *Econea* dermal and inhalation route exposures is 300 [i.e., a margin of exposure (MOE) less than 300 indicates potential risk concerns] for occupational scenarios. This LOC is based on uncertainty factors of 10x interspecies extrapolation, 10x intraspecies variation, and 3x for data gaps where a LOAEL was selected for lack of a NOAEL.

A summary of dose levels and endpoints selected for use in human risk assessments of *Econea* are presented below in Table 6. Endpoints for adult dermal and inhalation routes of exposure will be used in this assessment.

Table 6. Summary of Toxicological Doses and Endpoints for *ECONEA™ Technical* for Use in Human Risk Assessment

Exposure Scenario	Dose Used in Risk Assessment	Target MOE for Risk Assessment	Study and Toxicological Effects
Acute and Chronic Dietary	Risk assessment not required based on use pattern		
Non-Dietary Occupational/Residential Exposures			
Incidental Oral Short-Term (1-30 days) and Intermediate-Term (1–6 months) (Occupational /Residential)	NOAEL = <6.3 mg/kg/day (LDT) for females	Target MOE = 300 (10x interspecies extrapolation, 10x intraspecies variation, 3x for lack of a NOAEL in females)	Subchronic (90 Day) Oral - Rat LOAEL = 6.3 mg/kg/day (LDT) for females based on microscopic findings of the brain and spinal cord. *Classification: Acceptable/Guideline*
Dermal* Short-Term (1-30 days) and Intermediate-Term (1–6 months) (Occupational)	NOAEL = <6.3 mg/kg/day (LDT) for females	Target MOE = 300 (10x interspecies extrapolation, 10x intraspecies variation, 3x for the NOAEL data gap	Subchronic (90 Day) Oral - Rat LOAEL = 6.3 mg/kg/day (LDT) for females based on microscopic findings of the brain and spinal cord. *Classification: Acceptable/Guideline*
Dermal* Long-Term (6 months – lifetime) (Occupational)	Risk assessment not required based for this use pattern		

Table 6. Summary of Toxicological Doses and Endpoints for *ECONEA™ Technical* for Use in Human Risk Assessment			
Exposure Scenario	**Dose Used in Risk Assessment**	**Target MOE for Risk Assessment**	**Study and Toxicological Effects**
Inhalation ** Short-Term (1-30 days) and Intermediate (1 – 6 months) Term (Occupational)	**NOAEL** could not be calculated (<20mg/m3) (LDT). [Since toxicity was due to point-of-entry effects (i.e., local irritation of the dorsal region of the nose), an **Inhalation RfD** was estimated as 5.7 mg/kg/day.]	Target MOE = 300 10x interspecies extrapolation, 10x intraspecies variation, 3x conversion of LOAEL to NOAEL	**Subchronic (90 Day) Inhalation Toxicity – Rat (Phase I)** **LOAEL** is 20-mg/m^3 based on the findings observed in the nasal section NI to NO and included chronic and subacute inflammation , ulcerations, exudates, epithelial hyperplasia, hyperkeratosis and degeneration of the olfactory epithelium. Decreased body weight gain was also seen at this dose level. *Classification: Acceptable/Guideline*
Inhalation Long-Term (6 months – lifetime) (Occupational)	Risk assessment not required based for this use pattern.		
Cancer	No cancer data available.		

Source: Table based on AD/RASSB memorandum of June 29, 2006 from S.L. Malish and J. Chen to N. Cook, *"Toxicity Study Review of Econea-Technical and Toxicology Endpoints for Risk Assessment"* (D323129, D327538, D328778, and D330458).
NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, LDT = lowest dose tested, and MOE = margin of exposure.
* Dermal – 100% dermal absorption factor will be used.
** Inhalation – Where an Inhalation Reference Dose (RfD) is needed the following conversion equation was used: Estimated Inhalation RfD (mg/kg/day) = (Toxic concentration 20-mg/m^3 x Adult daily inhalation rate 20 m^3/day) / Adult body weight 70 kg. This approach assumes that the same air concentration would cause equivalent effects in both rat and human receptors. (Guidance from J. Chen based on U.S. EPA, 1989 & 1994.)

4.0 OCCUPATIONAL EXPOSURE ASSESSMENT

4.1 Occupational Exposure/Risk Pathway

Sigma Nexxium 20 Antifouling is a ready-to-use paint containing 3.4% *Econea*. The label limits the applications to commercial applicators in commercial or government shipyards. The labeling states that at a minimum a PAPR respirator jointly approved by the Mine Safety and Health Administration and the National Institute for Occupational Safety and Health (NIOSH) must be worn while spraying paint, sanding, or sandblasting boat surfaces. The labeling indirectly recommends gloves (technical data sheet) but does not instruct painters to wear protective clothing such as protective eyewear (safety glasses, goggles or face shield), chemical resistant gloves, long sleeved cotton shirt, long pants, and hat. ***Sigma Nexxium 20 Antifouling*** is applied at 220 sq. ft/gallon as the optimal spreading rate (dry film thickness of 4 mils). The labeling cites an overcoating interval of 8 hours minimum, so only one full coat is assumed to be applied per day.

Based on the use patterns specified for ***Sigma Nexxium 20 Antifouling***, AD/RASSB has determined that there is a potential for exposures to mixers, loaders, applicators, or other handlers associated with the new active ingredient, *Econea*. Table 7 presents the exposure scenarios assessed in this document.

Table 7. Shipyard Exposure Scenarios Associated with Occupational Handlers of *Econea* in *Sigma Nexxium 20 Antifouling*

Representative Use	Method of Application	Exposure Scenario	Application Rate (% a.i.)
Use Site Category: IX - Antifoulant Paints			
Antifoulant paint applications to boat hulls	Paint Tenders: • Open pour liquid mixing/loading	ST/IT : Dermal and Inhalation (Baseline and PPE)	3.4% (RTU)
	Paint Applicators: • airless sprayer (primary) • bush/roller (secondary)	ST/IT : Dermal and Inhalation (Baseline and PPE)	3.4% (RTU)

ST= short-term (1 to 30 days), IT=intermediate-term (1 to 6 months), RTU = ready-to-use formulation.

Industrial shipyard operations are closely regulated as an industry sector by The U.S. Department of Labor's Occupational Safety & Health Administration (OSHA). These include oversight of worker safety practices during painting operations, inclusive of antifoulants. OSHA has set compliance standards [1915.35] for clothing PPE and respiratory protection during shipyard spray applications and hand applications (brush or roller). In particular, powered air purifying respirators (PAPRs) are cited when ambient air ventilation is adequate, and air-line respirators (i.e., supplied-air respirators – SAR) are specified while painting/working in confined or enclosed spaces. Since limited PPE is specified on the ***Sigma Nexxium 20 Antifouling*** labeling (powered air purifying respirator- PAPR) and technical data sheet (gloves/fresh air mask) several PPE scenarios will be assessed. Typical industrial safety practices include the following PPE: Hood/head cover, safety glasses/goggles, appropriate respirator, coveralls, Tyvek paper suit, gloves, safety shoes and barrier cream for exposed facial skin as appropriate. Handlers review labels and Material Safety Data Sheets (MSDS) and comply with OSHA regulations (ACC, 2005).

4.2 Occupational Handlers Exposures and Risks

Chemical-specific worker exposure monitoring data were not submitted or cited in support of this new antifoulant (*Econea*) to assess exposures for industrial shipyard workers as painters and handlers of ***Sigma Nexxium 20 Antifouling*** paint. However, certain data were provided by Janssen Pharmaceutica, Inc. in the form of an occupational exposure report (the most recent version as MRID 468466-02) dated May 15, 2006,

entitled "*Revision 1 to MRID No. 46751303: Screening Level Occupational Exposure Assessments For R107894 (AC or CL 303268) As An Antifoulant In Paint Applied To Underwater Hulls of Pleasure, Military, and Commercial Craft.*" The assessments are intended to evaluate the potential worker exposures during commercial painting operations. As a conservative screening tool the submitted report included qualitative observations of shipyard workers and quantitative dermal/inhalation exposure estimates and calculated MOEs for different painter scenarios (i.e., paint mixer/loader/applicator scenarios) based on surrogate data from the Pesticide Handlers Exposure Database (PHED).

An evaluation of non-dietary occupational exposures was conducted by AD/RASSB on representative scenarios for exposure routes of concern based on the ***Sigma Nexxium 20 Antifouling*** product labeling use patterns and the toxicity of the active ingredient. (See Table 8.) No chemical-specific exposure data are available. Therefore, the assessment relied on certain Agency standard values and surrogate data sources to develop scenarios; including surrogate unit exposure data taken from PHED, Version 1.1 (U.S. EPA, 1998). Certain registrant-submitted information on shipyard worker observations (MRID 468466-02) was used to aid in calculating handler doses at baseline and when PPE is worn:

Shipyard Workers Engaged in Boat Hull Painting Operations

The assessment presented is based on label specifications for ***Sigma Nexxium 20 Antifouling*** :

- the mass density of the product (12.51 lb/gal) and
- spreading rate (220 sq.ft/gal) as optimal at 4 mils dry film thickness
- 3.4% a.i., Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-5-(trifluoromethyl) (*R107894*, PC Code:119093)

The submitted data (MRID 468466-02) cites the following painting operation observations. These observations were presented based on shipyard activities. When boats are drydocked, the hulls are high-pressure washed with water, sanded or grit-blasted as necessary to remove old coatings, coated with primers, then painted with antifoulants (and other coatings as needed). Actual antifoulant painting activities represent a small fraction of the overall time spent in refurbishing drydocked vessels:

- *Paint Tenders:* 1-3 paint stations/worker will be tended for paint applicators so it is estimated that they handle 3X the amount of paint (a.i.) as a painter. 5-gal pails of paint are handled from pallets and taken to pressure units where thinners may be added and paint mixing conducted using an electric-powered agitator. Coatings are poured (open pour) from pail-to-pail. Typical PPE is Tyvek coveralls over street clothes; leather safety boots; hard hats; eye protection; and heavy rubber gloves. (*Note: no respiratory protection.*)

- *Paint Applicators (Airless Sprayer):* The paint station consists of the pressure unit for the airless spraygun. A take-up hose in a can of paint supplied the pressure unit, which sent paint through a 30 meter hose to the handheld spraygun. The painter (applicator) worked from the floor or from the basket of a man-lift. PPE in addition to the standard safety boots and hard hats, typically included a range of clothing ensembles based on the coating being applied. For an antifoulant, clothing may include long pants and long-sleeved shirts to full PPE: Tyvek coveralls; woven fabric hood under a hard hat; eye protection; respirator; and gloves. "During a normal work shift, a worker will spend approximately 6 hours painting. He will handle the most paint when the entire hull is being coated to 4 mils, on average 500 sq. ft. per hour."

- Unit Exposures are from PHED where the following PPE and corresponding protection factors were incorporated:

- long pants, long sleeved shirts (baseline)
- additional layer of clothes as in Tyvek coveralls (50% protection factor),
- chemical resistant gloves (90% protection factor when no gloved data are available),
- dust/mist respirator (80% protection factor)
- organic vapour respirator (90% protection factor).

- Other protection factors applied are the assigned protection factors (APF) from OSHA's National Institute for Occupational Safety and Health (NIOSH) for powered air purifying (PAPR) and supplied air (SAR) respirators (NIOSH, 2004):

- PAPR-full-facepiece /SAR-full-facepiece respirator (50X protection factor as $1/50^{th}$ of airborne contaminants inhaled)
- SAR-full-facepiece pressure demand (1000X protection factor as $1/1000^{th}$ of airborne contaminants inhaled).

Assumptions and factors used in the shipyard scenario assessments detailed in Table 8 are described below:

- **Unit Exposures (UE):** ***Shipyard Antifoulant Painters (Applicators)***
Dermal and inhalation unit exposure values were taken from the Pesticide Handler Exposure Database. Antifoulant paints are applied primarily by airless sprayers in shipyards and dry docks (ACC, 2005). Therefore, for professionals applying *Econea*-based antifoulant paint to ships, the PHED dermal and inhalation unit exposure values for ***airless sprayer*** applications (PHED scenario 23) were utilized in this assessment (USEPA, 1998). The dermal unit exposure is 38 mg/lb a.i. for ungloved replicates (single layer of clothing) and 14.34 mg/lb a.i. for gloved replicates. A scenario was added for the addition of an additional layer of clothes

as Tyvek coveralls (50% protection factor to torso) to cover body as 13.6 mg/lb ai.

- The PHED inhalation unit exposure value for the ***airless sprayer*** application was available in terms of an air concentration (mg/m^3/% a.i.) as well as, in terms of amount handled (mg/lb a.i.). Since the inhalation toxicity endpoint was determined from an inhalation study (as opposed to an oral study), the endpoint units are given in terms of an air concentration (mg/m^3). Therefore, in order to more accurately estimate inhalation risks (MOEs), it was more appropriate to use the unit exposure value in terms of an air concentration (mg/m^3/% a.i.) rather than amount handled (mg/lb a.i.) (See Appendix A). Furthermore, although the label does require the use of a PAPR class respirator, it does not specifically identify the type of PAPR respirator (e.g., full-facepiece or hood/helmet) to be worn. Nor is there mention of special respiratory protection for work in enclosed or confined spaces (i.e., OSHA specified SAR respirators). Therefore, this assessment evaluated other PPE scenarios in addition to the baseline (i.e., no respirator) inhalation exposures; including PHED 90% PF for organic vapour respirator (10X); a PAPR(or SAR) full-face respirator that provide a 50X protection factor, and a SAR full face respirator with positive pressure that provides a 1000X protection factor (NIOSH, 2004). The inhalation unit exposure values are 0.681 mg/m^3/% a.i for baseline, 0.0681 mg/m^3/% a.i for PHED respirator, 0.0136 mg/m^3/% a.i for a PAPR(SAR) full face respirator, and 0.00068 mg/m^3/% a.i for a SAR full face respirator with positive pressure.

- Shipyard painters use ***brush and roller*** equipment for touch-up and repair as minor painting techniques. There are no chemical-specific exposure data to assess these techniques, however surrogate PHED data are available for painting with a brush. The surrogate data are based on painters wearing long pants, long sleeve shirts, no gloves, and no respirator. The PHED test subjects were monitored while painting a bathroom with a paint brush. Although the exposures while painting a boat hull will differ, these data are judged to be adequately representative. For the ***brush and roller*** applications, dermal and inhalation unit exposure were taken from PHED Scenario 22. The dermal unit exposure is 180 mg/lb a.i. for ungloved replicates (single layer clothing; long-sleeved shirt, long pants; no gloves) and 24 mg/lb a.i. with gloves. A scenario was added for the addition of an additional layer of clothes as Tyvek coveralls (50% protection factor to torso) yielding 22 mg/lb a.i.. The inhalation unit exposure is 0.28 mg/lb a.i. (no respirator). Baseline inhalation exposures (i.e., no respirator) as well as exposures wearing a PHED dust/mist respirator which provides 80% protection (20X), a PHED organic vapour respirator that provides a 90% protection factor (10X), and a PAPR(or SAR) full-face respirator that provide a 50X protection factor (NIOSH, 2004). The inhalation unit exposure values are 0.28 mg/lb a.i. for baseline, 0.056 mg/lb a.i. for a PHED dust/mist, 0.028 mg/lb a.i. for PHED vapour respirator, and 0.0056 mg/lb a.i. for a PAPR(or SAR) full-face respirator.

- **Unit Exposures (UE):** ***Shipyard Antifoulant Paint Tenders (Mixer/Loader)*** For paint tenders, the most representative surrogate unit exposure data available is PHED Scenario 3, ***open mixing/loading*** of all liquids. The label does not specify mandatory use of gloves when pouring and blending the paints, so this assessment evaluated workers with and without (i.e., baseline) gloves. The dermal unit exposures are 2.9 mg/lb a.i. for ungloved replicates and 0.023 mg/lb a.i. for gloved replicates. A scenario was added for the addition of an additional layer of clothes as Tyvek coveralls (50% protection factor to torso) yielding 0.01748 mg/lb a.i.. Furthermore, although the label does require the use of a PAPR while spray painting, it does not specify respiratory protection for ancillary workers engaged in paint mixer/loader tasks (i.e., paint tenders). Therefore, this assessment evaluated the baseline inhalation exposures (i.e., no respirator) as well as exposures wearing a PHED dust/mist respirator which provides 80% protection (20X), a PHED organic vapour respirator that provides a 90% protection factor (10X), and a PAPR(or SAR) full-face respirator that provide a 50X protection factor (NIOSH, 2004). The inhalation unit exposure values are 0.0012 mg/lb a.i. for baseline, 0.00024 mg/lb a.i. for a PHED dust/mist, 0.00012 mg/lb a.i. for a PHED vapour respirator, and 0.000024 mg/lb a.i. for a full face respirator.

- **Amount handled:** Based on AD's standard assumptions and knowledge of shipyard painting practices, a shipyard painter over the course of a day may handle upwards of 50 gallons of paint via airless sprayer depending on the coating and desired film thickness (industry estimate ACC, 2005). However, based on submitted observational data (MRID 468466-02) the ST/IT assessment assumed that a shipyard painter will handle closer to 14 gallons of paint using an ***airless sprayer*** for 3000 sq ft of coverage (observations of 500 sq ft/hr x 6 hr/day x gal/220 sq ft = 14 gallons applied at a dry film thickness of 4 mils). For ***brush and roller***, as a minor painting scenario, Agency standard estimates of 5 gals/day were used (or 63 lbs/day, assuming paint has a density of 12.51 lbs/gal) equating to 1100 sq ft of coverage at a maximum. For ***open mixing/loading***, paint tenders handle 3X the amount of paint as do airless spray painters. Therefore 42 gallons were estimated. (3 x 14 gals for airless = 42 gallons/day).

- **Exposure time:** (For *airless sprayer* scenario only.) As previously mentioned, the inhalation unit exposure for the airless sprayer was provided in terms of an air concentration (mg/m^3/% a.i.) due to the use of an inhalation (not oral) toxicity study. Since the inhalation toxicity study was based on the animals being exposed to the chemical for 6 hours per day, the worker exposure and MOE equation had to be modified to account for the amount of time that the worker is actually exposed to the active ingredient. In this case, it was assumed that it would take a professional shipyard painter 6 hours to accomplish this task using an airless sprayer (MRID 468466-02, and ACC, 2005).

Table 8. ST/IT Exposures and MOEs for Shipyard Painters of *Sigma Nexxium 20 Antifouling* Paint

Scenario	Application Rate (% ai)	Dermal UE (mg/lb ai)	Dermal PPE	Inhalation Air Conc UE (mg/m3/%ai)	Respiratory PPE	Amount Handled (lb/day) [a]	Dermal Dose (mg/kg/day) [b]	Dermal MOE [c] Target = 300	Exposure Time (hr/day)	Inhalation Air Conc MOE [d] Target = 300
Airless sprayer	3.4% *Econea*	38	Baseline	0.681	Baseline	175	3.23	2	6.0	7
		14.34	Gloves	0.0681	PHED Respirator	175	1.22	5		60
		13.6	Tyvek coveralls	0.0136	PAPR-face resp	175	1.2	5		400
		--	--	0.00068	SAR-Full-face resp. w/ pos. pressure	175	--	--		6000
Scenario	**Application Rate (% ai)**	**Dermal UE (mg/lb ai)**	**Dermal PPE**	**Inhalation UE (mg/lb ai)**	**Respiratory PPE**	**Amount Handled (lb/day) [a]**	**Dermal Dose (mg/kg/day) [b]**	**Dermal MOE [c]**	**Inhalation Dose [b] (mg/kg/day)**	**Inhalation MOE [c]**
Brush & Roller	3.4% *Econea*	180	Baseline	0.28	Baseline	63	6	1	0.0086	663
		24	Gloves	0.056	PHED Dust/Mist	63	0.734	9	0.002	2850
		22	Tyvek coveralls	0.028	PHED Respirator	63	0.673	9	0.0009	6333
		--	--	0.0056	PAPR-face resp	63	--	--	0.0002	28,500
Paint Tenders: (open pour mix/load)		2.9	Baseline	0.0012	Baseline	525	0.74	9	0.0003	19,000
		0.023	Gloves	0.00024	PHED Dust/Mist	525	0.006	1050	0.00006	95,000
		0.01748	Tyvek coveralls	0.00012	PHED Respirator	525	0.0045	1450	0.00003	190,000
		--	--	0.000024	PAPR-face resp	525	--	--	0.000006	950,000

Note: UE = Unit Exposure, as derived from PHED Version 1.1, August, 1998 "PHED Surrogate Exposure Guide". PHED values as described within this review.
PHED values for Dermal/Inhalation UE and PPE: Baseline Dermal = "Single Layer, No Gloves" to represent a single layer of work clothes (long-sleeve shirt, long pants) and no protective gloves; Dermal PPE = the use chemical-resistant gloves and coveralls added to the baseline clothing scenario. Baseline Inhalation = No respirator used; Respiratory PPE = Use of a specified respirator: PHED Dust/Mist, PHED Respirator (organic vapour), PAPR full-face, and SAR full-face with positive pressure.
a: lb/day = gal/day x 12.51 lb/gal (paint density): 14 gallons Airless; 5 gallons Paint Brush; 42 gallons Paint tenders.
b: Dose (mg/kg/day) = app rate (%ai) x UE (mg/lb ai) x quantity handled (lb/day) / BW (70 kg)
c: MOE = ST/IT NOAEL (mg/kg/day) / Dose (mg/kg/day) where dermal ST/IT NOAEL = 6.3 mg/kg/day and inhalation ST/IT NOAEL = 5.7 mg/kg/day. Target MOE = 300
d: air conc inhalation MOE = (ST/IT NOAEL of 20 mg/m^3 x animal exposure time 6 hrs/day) / [(UE mg/m^3/% ai x % ai (not fraction a.i.) x worker exposure time hr/day) x (1.25 m^3 per hour inhalation rate)].

4.3 Occupational Postapplication Exposure

Occupational postapplication exposures were not assessed. No exposure data have been submitted to the Agency to determine the extent of postapplication exposures for worker scenarios. It is assumed that exposures in industrial settings following painting operations will be less than handler exposure during painting. Inhalation concerns post-treatment should be minimal due to the non-volatile nature of the active ingredient (1.9×10^{-8} kPa at 20°C) and the lack of aerosol/spray mist generation once boat hull painting is done. *Econea* is unlikely to volatilize appreciably at room temperature during clean-up tasks. Dermal contact with painted surfaces is also not a concern since workers do not remain in work areas or re-enter as fresh paint is drying.

5.0 ASSESSMENT OF DATA GAPS AND UNCERTAINTIES

The non-dietary occupational exposure assessment was conducted for the new antifoulant active ingredient *Econea*, in the end-use paint product, ***Sigma Nexxium 20 Antifouling*** to identify any potential exposure concerns associated with this new pesticide. The following items are some of the data limitations and uncertainties for this assessment:

- Chemical-specific exposure monitoring data were not available; therefore as is policy, the Agency used surrogate data sources and standard approaches which may not realistically estimate exposure during actual use conditions. Surrogate unit exposure values were taken from the Pesticide Handlers Exposure Database (PHED) (USEPA, 1998). (See Appendix A for a summary of PHED data).

- AD/RASSB relied upon professional judgment of industrial practices, product labeling use rates/methods and certain registrant-provided inputs (taken from submitted data MRID 468466-02) for estimating quantity handled/day and surface area painted/day. It is not known if the observational data for shipyard painting operations (MRID 468466-02) may actually underestimate the quantity handled/area painted (e.g., application rate of 500 sq ft/hr and 3000 sq ft/day painted surface area).

- The dermal-route exposure risks were based on a toxicological endpoint from a 90-day oral toxicity study in the rat (i.e., the NOAEL is <6.3 mg/kg/day). It should be noted that the registrant had conducted a 90-day dermal study in the rat (MRID 46802201) which was deficient for lack of performed lung histopathology, but upgradeable. The available study data indicates a dermal NOAEL of 100 mg/kg/day and a systemic NOAEL of 300 mg/kg/day. These data can be upgraded if lung pathology is evaluated to note the frequency of lung lesions at the low and intermediate dose levels to better define the NOAEL/LOAEL. If the dermal study is used for toxicological hazard, and the 3X database uncertainty factor removed, then several occupational scenarios which triggered dermal risk concerns will yield more favorable outcomes.

6.0 REFERENCES

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cc: Doreen Aviado/RASSB/AD
Chemical File
Circulation File

APPENDIX A: Summary of PHED data

The Pesticide Handlers Exposure Database (PHED):

The Pesticide Handlers Exposure Database (PHED) has been developed by a Task Force consisting of representatives from Health Canada, the U.S. Environmental Protection Agency (EPA), and the American Crop Protection Association (ACPA). PHED provides generic pesticide worker (i.e., mixer/loader and applicator) exposure estimates. The dermal and inhalation exposure estimates generated by PHED are based on actual field monitoring data, which are reported generically (i.e., chemical specific names not reported) in PHED. It has been the Agency's policy to use "surrogate" or "generic" exposure data for pesticide applicators in certain circumstances because it is believed that the physical parameters (e.g., packaging type) or application technique (e.g., aerosol can), not the chemical properties of the pesticide, attribute to exposure levels. [Note: Vapor pressures for the chemicals in PHED are in the range of E-5 to E-7 mm Hg.] Chemical-specific properties are accounted for by correcting the exposure data for study specific field and laboratory recovery values as specified by the PHED grading criteria.

PHED handler exposure data are generally provided on a normalized basis for use in exposure assessments. The most common method for normalizing exposure is by pounds (lbs) of active ingredient (ai) handled per replicate (i.e., exposure in mg per replicate is divided by the amount of ai handled in that particular replicate). These unit exposures are expressed as mg/lb ai handled. This normalization method presumes that dermal and inhalation exposures are linear based on the amount of active ingredient handled.

For the assessment of Shipyard workers using airless spraying techniques to apply *Econea*-based antifoulant paints, the PHED data for the Airless Sprayer scenario (Scenario 23) was used. Since a route-specific inhalation toxicity endpoint for *Econea* was selected for determining risk, the normalized inhalation unit air concentration (0.681 mg/m3/%ai) from PHED was used for exposure dose calculations. Attached are the raw data for the air concentrations in PHED study 0467 during airless sprayer application (exterior house stain containing 0.5% ai fungicide applied to a residence). The air concentrations are reported in this spreadsheet as monitored in the study (mg/m3) as well as "unit exposures (UE)" normalized to lb ai handled (mg/m3/lb ai) and also normalized by % ai in the stain (mg/m3/%ai). They are actually "unit air concentrations", not "exposures". The % ai in the final treatment solution/antifoulant coating is used as the normalization variable rather then lb ai handled (i.e., mg/m3/%ai).

PHED Airless Sprayer Study 0467 Data -- (exterior house stain application to residential home 0.5% a.i. fungicide)

Record I.D.	Total AI applied (lb)	Volume handled (gallons)	Final Conc (lb ai/gal)	Treatment Solution (% ai)	Air Sample Time (min)	Spray Rate (gal/hour)	Air Amount (ug)	Air LOQ (ug)	Air Volume (liter)	Average Flow Rate (l/min)	Air Conc. Study (ug/L)	Air Conc. Study (mg/m3)	Air Conc. Normalized (mg/m3/lb ai)	Air Conc. Normalized (mg/m3/%ai)
0467*C*01	0.1667	5	0.03334	0.5	17	17.6	4.3	2	34	2	0.126	0.126	0.759	0.253
0467*E*01	0.1667	5	0.03334	0.5	25	12.0	13.6	2	50	2	0.272	0.272	1.632	0.544
0467*G*01	0.1667	5	0.03334	0.5	20	15.0	10	2	40	2	0.250	0.250	1.500	0.500
0467*I*01	0.1867	5	0.03334	0.5	16	18.8	14.4	2	32	2	0.450	0.450	2.699	0.900
0467*K*01	0.1667	5	0.03334	0.5	18	18.7	13.7	2	36	2	0.381	0.381	2.283	0.761
0467*O*01	0.1667	5	0.03334	0.5	13	23.1	13.7	2	28	2	0.527	0.527	3.161	1.054
0467*A*01	0.1667	5	0.03334	0.5	27	11.1	8.8	2	54	2	0.163	0.163	0.978	0.326
0467*M*01	0.1667	5	0.03334	0.5	16	18.8	4.4	2	32	2	0.138	0.138	0.825	0.275
0467*B*01	0.1667	5	0.03334	0.5	13	23.1	20.1	2	26	2	0.773	0.773	4.638	1.546
0467*F*01	0.1667	5	0.03334	0.5	17	17.6	5.4	2	34	2	0.159	0.159	0.953	0.318
0467*H*01	0.1667	5	0.03334	0.5	11	27.3	5.5	2	22	2	0.250	0.250	1.500	0.500
0467*J*01	0.1667	5	0.03334	0.5	11	27.3	12.8	2	22	2	0.582	0.582	3.490	1.164
0467*D*01	0.1667	5	0.03334	0.5	21	14.3	16.8	2	42	2	0.400	0.400	2.400	0.800
0467*L*01	0.1667	5	0.03334	0.5	12	25.0	7.2	2	24	2	0.300	0.300	1.800	0.800
0467*N*01	0.1667	5	0.03334	0.5	13	23.1	8.7	2	26	2	0.335	0.335	2.007	0.669
Geometric mean	0.1667	5	0.033	0.5	16.0	18.7	9.5	2	32.1	2	0.297	0.297	1.783	0.594
Arithmetic mean	**0.1667**	**5**	**0.033**	**0.5**	**16.7**	**19.4**	**10.6**	**2**	**33.3**	**2**	**0.340**	**0.340**	**2.041**	**0.681**
std	5.7E-17	0	0.000	0.0	4.9	5.2	4.8	0	9.8	0	0.184	0.184	1.101	0.367
median	0.1667	5	0.033	0.5	16.0	18.8	10.0	2	32.0	2	0.300	0.300	1.800	0.600
75%tile	0.1667	5	0.033	0.5	19.0	23.1	13.7	2	38.0	2	0.425	0.425	2.549	0.850
90%tile	0.1667	5	0.033	0.5	23.4	26.4	15.8	2	46.8	2	0.560	0.560	3.358	1.120
maximum	0.1667	5	0.033	0.5	27.0	27.3	20.1	2	54.0	2	0.773	0.773	4.638	1.546

Record I.D.= Record identification from PHED study code 0467. Airless sprayer (3000 psi).
Volume handled represents 5 gallons of house stain sprayed. 5 gal covered 750 to 1250 sq. ft.
Final Concentration (lb ai/gal) = actual concentration used during the study (total lb ai / gallons sprayed).
Treatment solution (% ai) = 0.5% ai as reported in PHED study code 0467 (fungicide in house stain).
Air sample time (minutes) = the exposure time for each replicate in PHED study 0467 that the personal air pump ran.
Spray rate (gal/hour) = volume sprayed (5 gal) / (exposure duration in minutes/60 min per hour).
Air amount (ug) = amount of chemical analyzed on each air sampler.
Air LOQ (ug) = limit of quantification for air samplers, note: all values were detectable.
Air volume (liters) = volume of air sampled per replicate.
Average flow rate (l/min) = personal air samplers run at 2 l/min.
Air concentration in study = air amount (ug) / Air Volume (L); equivalent to mg/m3; Air Concentration normalized (mg/m3/lbai) = Air concentration in study / lb ai handled per replicate.
Air Concentration normalized (mg/m3/%ai) = Air concentration in study / % ai (0.5% ai used, not weight fraction of 0.005).

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

November 16, 2006

EPA United States Environmental Protection Agency Office of Pesticide Programs

MEMORANDUM

SUBJECT: Estimated Environmental Concentrations (EECs) for ECONEA ™ Antifouland Agent

From: Siroos Mostaghimi, Senior Scientist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

James Breithaupt, Agronomist
Environmental Risk Branch II
Environmental fate and Effects Division (7507P)

To: Marshall Swindell, PM 33
Regulatory Management Branch I (RMBI)
Antimicrobials Division (7510P)

Thru: Norm Cook, Chief
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

DP Barcode: 330548 and 330550
Pesticide Chemical No.: 119093
Registrant: Janssen Pharmacetuica Inc.

The following report contains a summary of the results from modeling data which were submitted by the Janssen Pharmacetuica Inc. in a submission titled "Environmental and Ecological Risk Assessment of ECONEA Antifoulant Agent (MRID# 468466-03)" . The inputs used for running MAM-PEC (Marine Antifoulant Model to predict Environmental Concentrations) and, EFDC (Environmental Fluid Dynamic Code), appear correct and the data reported from the runs are acceptable. The inputs for the TRIM2D (Tidal Residual Inter-tidal Mudflat) appear correct; however, the outputs from this model run could not be verified independently because of the licensing issues and the lack of availability of TRIM2D algorithms to the public.

Discussion and Conclusion

RASSB concludes that the data submitted by the registrant for modeling runs of MAMPEC and FEDC are acceptable and appear to be scientifically sound. However, the data from the TRIM2D could not be verified.

MAM-PEC is used as an assessment tool for antifoulant risk assessments in Europe. MAM-PEC was developed by the Institute of Environmental Studies/IVM and Delft Hydraulics for the European Paint Makers Association (CEPE) for conducting risk assessments for antifouling agents. The model provides prediction of environmental concentrations of antifouling products in six generalized "typical" marine environments (commercial harbor, estuarine harbor, marina, marina poorly flushed, open sea, and shipping lane).

FEDC is a multifunctional surface water modeling system, which includes hydrodynamic, sediment-contaminant, and eutrophication components. The EFDC model is capable of 1, 2, and 3-D spatial resolution. The model uses a curvilinear-orthogonal horizontal grid and a sigma terrain following vertical grid. The EFDC model can represent the transport and fate of an arbitrary number of contaminants, including metals and hydrophobic organics, sorbed to any of the sediment classes and dissolved and particulate organic carbon using a three-phase equilibrium partitioning formulation. The public domain EFDC program was originally developed at the Virginia Institute of Marine Science and is currently maintained by Tetra Tech, Inc. with support from the US EPA.

TRIM2D is a 2-dimensional, depth-averaged, finite-difference hydrodynamic model for simulating inland water flows governed by tidal, wind and riverine inputs. The model uses a high-resolution uniform grid to solve the incompressible Navier-Stokes equations. Simulation output includes water velocities, water surface elevations, salinity profiles, and the distribution of any released contaminants. The TRIM2D software was developed by the Space and Naval Warfare System Center San Diego (SSC SD), within the Department of the Navy, in collaboration with the U.S. Geological Survey (USGS). The algorithm for this model is not available to the public.

The active ingredient in ECONEA™ is R107894 (1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-trifluoromethyl), also known as CL303268. R107894 degrades rapidly to three metabolites (CL322250, CL322348 and CL325195).

R107894 breaks down rapidly in the environment. Degradation from aqueous hydrolysis has been reported to occur with half-lives of 3 and 15 hours in seawater (at temperatures of 25° and 10° C, respectively), and 2 and 12 hours in freshwater at pH 7 (25° and 10°C, respectively). Half-lives of 2 to 4 days in water have been reported in marine and freshwater aerobic aquatic metabolism studies. Half-lives in sediment or full test system were longer in those studies (31 and 13 days, respectively).

Degradation products include CL322250 and CL325195. CL322250 breaks down further to form CL322248. Maximum formation (percent of R107894) observed in marine aerobic aquatic metabolism studies have been 70, 76, and 7 percent for CL322250, CL322248, and CL325195, respectively.

The study submitted by the registrant focuses on CL322250 and CL322248 based on their expected respective rates of formation, persistence, toxicity, and potential for toxicological effects in the environment. R107894 is not addressed because of its rapid degradation in the environment and low potential for bioaccumulation. CL322195 is not addressed based on its relatively low rate of formation and low toxicity to test species.

Model simulations were used to estimate the concentrations of the CL322250 and CL322248 in five harbor system in the United States. The systems modeled, models used and the rationale for use of the models are presented in the Table 1.

Table1. Models used for estimating environmental concentrations of ECONEA ™ in different systems.

System	Model	Rationale
Commercial, Estuarine, Marina, Marina Poorly Flushed, Shipping Lane, and Open Sea	MAM-PEC	Screening level assessment using standard environments developed for the European Union.
Barbours Cut – Houston	MAM-PEC	Screening level representation of harbor system developed for this study.
Baltimore Harbor	MAM-PEC	Screening level representation of harbor system developed for this study.
Norfolk Harbor/James River	EFDC	Detailed representation of harbor system previously setup by VIMS.
Port of New Orleans, lower Mississippi River	EFDC	Detailed representation of harbor system developed for this study.
San Diego Bay	TRIM2D	Detailed representation of harbor system previously developed by SSC SD.

Estimated Environmental Concentrations (EECs):

The estimated environmental concentrations for CL322250 and CL322248 from MAM-PEC runs in Baltimore and Barbarous Point Houston are shown in table 2. Both maximum and average concentration in water column and sediments are presented in this table.

Table2. Maximum and Average concentrations of CL322250 and CL322248 in Baltimore harbor and Barbours Point Houston estimated by MAM-PEC model.

Chemical	Statistics	Location			
		Baltimore		Barbours Point Houston	
		Water µg/l	Sediment (µg/g dw)	Water µg/l	Sediment (µg/g dw)
CL322250	Maximum	0.041	7.77E-5	0.448	8.44E-4
	Average	0.024	4.51E-5	0.335	6.32E-4
CL322248	Maximum	0.037	1.54E-4	0.406	1.66E-3
	Average	0.022	8.92E-5	0.304	1.24E-3

The EECs for CL322250 and CL322248 from the TRIM2D model run in San Diego Harbor are presented in Table 3. The chemical partitioning to sediments were not predicted by TRIM2D, therefore only concentrations in water are shown in this table.

Table 3 Maximum and Average concentrations of CL322250 and CL322248 in San Diego Harbor estimated by TRIMD2 model

Chemical	Statistics	Location
		San Diego harbor
		Water (µg/l)
CL322250	Maximum	3.840
	Average	1.816
CL322248	Maximum	4.174
	Average	2.173

The concentrations for CL322250 and CL322248 from the EFDC model results in Norfolk Harbor and Mississippi River are presented in Table 4. Both maximum and average concentration in water column and sediments are presented in this table.

Table4. Maximum and Average concentrations of CL322250 and CL322248 in Norfolk Harbor and Mississippi River estimated by EFDC model.

Chemical	Statistics	Location			
		Norfolk		Mississippi River	
		Water µg/l	Sediment (µg/g dw)	Water µg/l	Sediment (µg/g dw)
CL322250	Maximum	0.760	4.87E-3	0.233	<1.0E-10
	Average	0.180	4.05E-4	0.019	<1.0E-10
CL322248	Maximum	0.742	3.92E-4	0.211	<1.0E-9
	Average	0.0.188	7.85E-5	0.017	<1.0E-9

The estimated concentrations from the Mississippi River should be used for the dietary exposure assessment. The maximum concentrations should be used for short term and the average concentrations should be used chronic dietary assessment.

The data from MAP-PEC result for the Barbours Point Houston in water should be used for the ecological risk assessment. It should be noted that the highest concentrations were reported in San Diego Harbor by the TRIMD2 model. However, because of the lack of enough information for the TRIMD2 model the data from this model could not be verified independently.

File: C:\Myfiles\2006 Reports/ ECONEA Modeling\EECs for ECONEA

CC: Siroos Mostaghimi/RASSB
RASSB Chemical Files

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM:

Date: November 27, 2006

Subject: Drinking Water Exposure and Risk Assessments for the Registration Action of ECONEA™

To: Marshall Swindell, Product Manager 33
Regulatory Management Branch 1 (RMB1)
Antimicrobials Division (7510P)

From: Cassi L. Walls, Ph.D., Chemist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Thru: Norm Cook, Branch Chief
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Chemical No.: 119093

Chemical Name: 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-trifluoromethyl; R107894; CL303268

Action Requested:

The Risk Assessment and Science Support Branch (RASSB) was requested by Regulatory Management Branch 1 (RMBI) to conduct human health drinking water exposure and risk assessments for the active ingredient ECONEA™ (1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-trifluoromethyl), also known as R107894 and CL303268.

Discussion and Conclusion:

At this time, an accurate drinking water exposure and risk assessment is unable to be performed for the following reasons:

- ECONEA (CL303268) rapidly breaks down in the environment. The degradation products of CL303268 include CL322250 and CL325195. CL322250 further breaks down into CL322248. Based on their expected rates of formation, persistence, and aquatic toxicity, only CL322250 and CL322248 water concentrations were determined. ECONEA (CL303268) water concentration was not determined due to its rapid degradation in the environment and low potential for bioaccumulation. Furthermore, the water concentration for CL322195 was not determined due to its low rate of formation and low toxicity to aquatic test species. For a complete description of the determination of Estimation Environmental Concentrations (EECs) for ECONEA Antifoulant Agent, the reader is referred to DP 330548 and 330550 dated November 16, 2006.

- Currently, there are only human toxicological endpoints selected for the parent compound, ECONEA (CL303268), and no endpoints for the degradation products, CL322250 and CL322248. Furthermore, no acute and chronic dietary endpoints were selected for ECONEA. For a complete description of the determination of toxicological endpoints of ECONEA, the reader is referred to DP 323129 dated June 22, 2006.

- Since ECONEA rapidly degrades in the environment, it is reasonable to only determine the EECs for the degradation products. However, since no human toxicological endpoints are available for these break down products, it is impossible to conduct a human health drinking water risk assessment.

Decision #: 220066

DATA PACKAGE BEAN SHEET

DP #: (301745)

Date: 01-Dec-2006

* * * Registration Information * * *

Registration: 43813-ET - ECONEA TECHNICAL

Company: 43813 - JANSSEN PHARMACEUTICA INC.

Risk Manager: RM 33 - Marshall Swindell - (703) 308-6341 Room# PY1 S-8828

Risk Manager Reviewer: Norman Cook NCDDK

Sent Date: 19-Mar-2003 Calculated Due Date: 08-Aug-2006 Edited Due Date:

Type of Registration: Product Registration - Section 3

Action Desc: (A41) NEW AI;NDN-FDDD USE;DUTDDDR;DTHER USES;

Ingredients: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-(93.2%)

* * * Data Package Information * * *

Expedite: Yes ● No Date Sent: 26-Apr-2004 Due Back:

DP Ingredient: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-

DP Title:

CSF Included: Yes ● No Label Included: Yes ● No Parent DP #: 289021

Assigned To	Date In	Date Out	
Organization: AD / RASSB	26-Apr-2004	01-Dec-2006	Last Possible Science Due Date: 03-Jul-2006
Team Name: RASSB1	26-Apr-2004	01-Dec-2006	Science Due Date:
Reviewer Name: Walls, Cassi	23-Oct-2006	01-Dec-2006	Sub Data Package Due Date:
Contractor Name:			

* * * Studies Sent for Review * * *

No Studies

* * * Additional Data Package for this Decision * * *

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* * * Data Package Instructions * * *

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DATA PACKAGE BEAN SHEET

Decision #: 220066

DP #: (301745)

Date: 01-Dec-2006

* * * Registration Information * * *

Registration: 43813-ET - ECONEA TECHNICAL

Company: 43813 - JANSSEN PHARMACEUTICA INC.

Risk Manager: RM 33 - Marshall Swindell - (703) 308-6341 Room# PY1 S-8828

Risk Manager Reviewer: Norman Cook NCDDK

Sent Date: 19-Mar-2003 Calculated Due Date: 08-Aug-2006 Edited Due Date:

Type of Registration: Product Registration - Section 3

Action Desc: (A41) NEW AI;NON-FDDD USE;OUTDOOR;OTHER USES;

Ingredients: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-(93.2%)

* * * Data Package Information * * *

Expedite: Yes ● No Date Sent: 26-Apr-2004 Due Back:

DP Ingredient: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-

DP Title:

CSF Included: Yes ● No Label Included: Yes ● No Parent DP #: 289021

Assigned To	Date In	Date Out	
Organization: AD / RASSB	26-Apr-2004	01-Dec-2006	Last Possible Science Due Date: 03-Jul-2006
Team Name: RASSB1	26-Apr-2004	01-Dec-2006	Science Due Date:
Reviewer Name: Watts, Cassi	23-Oct-2006	01-Dec-2006	Sub Data Package Due Date:
Contractor Name:			

* * * Studies Sent for Review * * *

No Studies

* * * Additional Data Package for this Decision * * *

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* * * Data Package Instructions * * *

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